CLINICO-HEMATOLOGICAL PROFILE AND IRON STUDIES IN HAEMOPHILIA PATIENTS IN GOVT ROYAPETTAH HOSPITAL

Dissertation submitted to

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In partial fulfilment of regulations

For award of the degree of

M.D (GENERAL MEDICINE)

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CHENNAI 600 014

April 2015

BONAFIDE CERTIFICATE

This is to certify that dissertation named **"CLINICO-HEMATOLOGICAL PROFILE AND IRON STUDIES IN HAEMOPHILIA PATIENTS IN GOVT ROYAPETTAH HOSPITAL"** is a bonafide work performed by Dr.A.Ramya, post graduate student, Department of Internal Medicine, Kilpauk Medical College, Chennai-10, under my guidance and supervision in fulfilment of regulations of the Tamilnadu Dr. M.G.R Medical University for the award of M.D. Degree Branch I (General Medicine) during the academic period from May 2012to April 2015.

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DECLARATION

I solemnly declare that this dissertation **"CLINICO-HEMATOLOGICAL PROFILE AND IRON STUDIES IN HAEMOPHILIAPATIENTS IN GOVT ROYAPETTAH HOSPITAL"** was prepared by me at Government Kilpauk Medical College and Hospital, Chennai, under the guidance and supervision of **Dr. R.Sabaratnavel M.D.**, Professor, Department of Internal Medicine, Government Royapettah Hospital, Chennai.

This dissertation is submitted to **The Tamil Nadu Dr. M.G.R. Medical University, Chennai** in partial fulfilment of the University regulations for the award of the degree of **M.D. Branch I (General Medicine)**.

Place: Chennai

Date: (Dr. A.Ramya)

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CERTIFICATE OF APPROVAL

The Institutional Ethical Committee of Govt. Kilpauk Medical College, Chennai reviewed and discussed the application for approval "A Study of Clinico-Hematological profil and iron studies in hemophilia patients in Govt. Royapettah Hospital" – For Project Work submitted by Dr.A.Ramya, (10.08.1988), MD (GM), PG Student, KMC / GRH, Chennai.

The Proposal is APPROVED.

The Institutional Ethical Committee expects to be informed about the progress of the study any Adverse Drug Reaction Occurring in the Course of the study any change in the protocol and patient information /informed consent and asks to be provided a copy of the final report.

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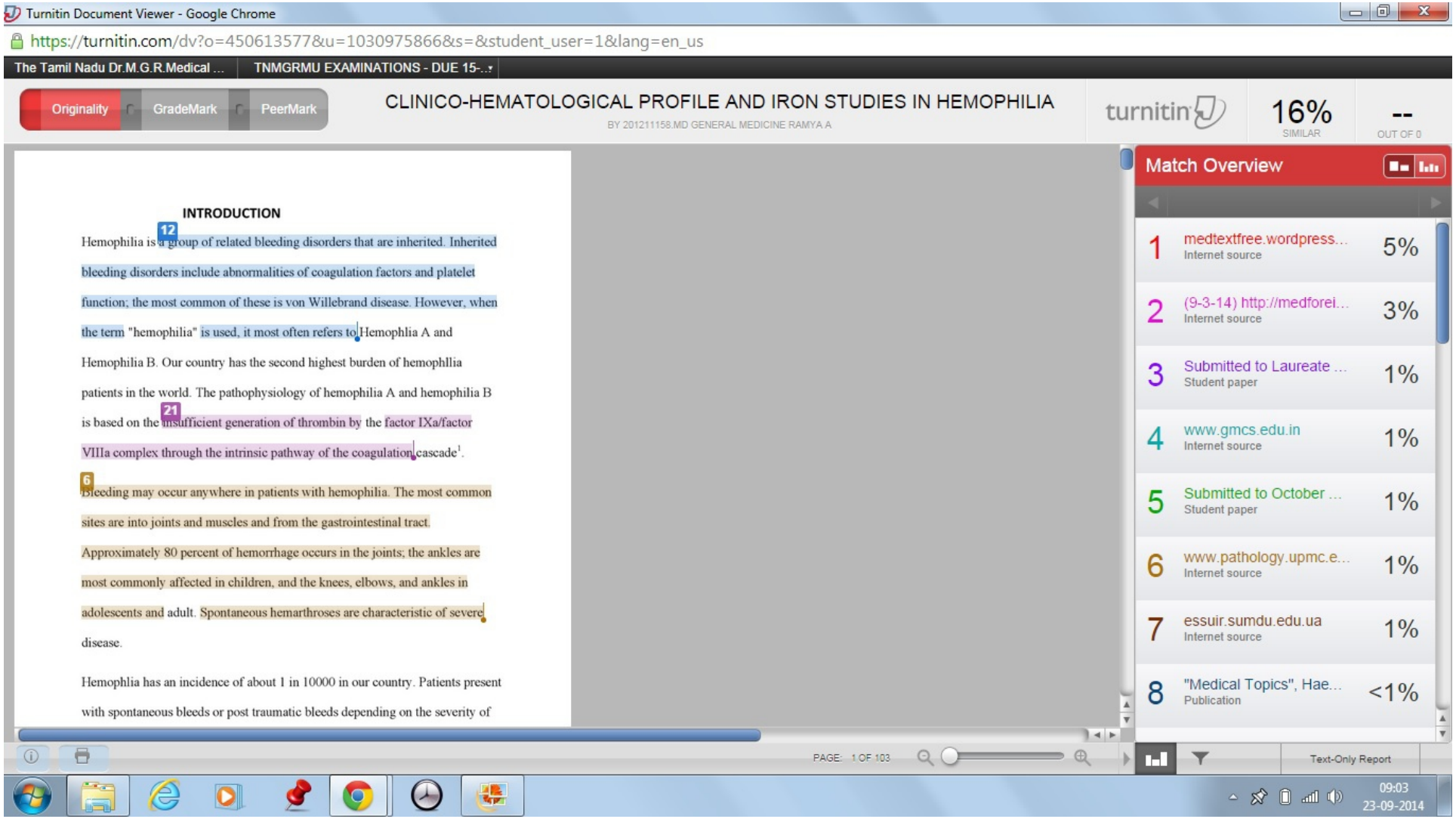
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INTRODUCTION
Hemophilia is a group of related bleeding disorders that are inherited. Inherited bleeding disorders include abnormalities of coagulation factors and platelet function; the most common of these is von Willebrand disease. However, when the term "hemophilia" is used, it most often refers to Hemophlia A and Hemophilia B. Our country has the second highest burden of hemophllia patients in the world. The pathophysiology of hemophilia A and hemophilia B is based on the insufficient generation of thrombin by the factor IXa/factor VIIIa complex through the intrinsic pathway of the coagulation cascade[1].
Bleeding may occur anywhere in patients with hemophilia. The most common sites are into joints and muscles and from the gastrointestinal tract. Approximately 80 percent of hemorrhage occurs in the joints; the ankles are most commonly affected in children, and the knees, elbows, and ankles in adolescents and adult. Spontaneous hemarthroses are characteristic of severe disease.
Hemophlia has an incidence of about 1 in 10000 in our country. Patients present with spontaneous bleeds or post traumatic bleeds depending on the severity of
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CONTENTS

ABSTRACT

BACKGROUND:

Haemophilia, an inherited single gene disorder has an incidence of 1 per 10,000 births. Although the genetic basis of this disorder has been well studied in India, data on the number of patients and trends of the disorder in India have not been reported.

METHODS:

A cross sectional study including 50 Haemophilia patients was done. After obtaining consent from the patients, a detailed clinical history and laboratory investigations, including factor assays and ferritin levels were done. Ferritin levels were measured by Immunoassay method.

RESULTS:

The results showed that severe haemophiliacs formed the major proportion of the patients. There were 64% of severe Hemophilia A and 8% were inhibitor positive. 42% had a family history of hemophlia. The mean age at diagnosis was 6.58 months. Arthropathy involved the weight bearing joints. Ferritin levels were found to be in the low normal range with 62.5% of severe Hemophilia A patients having a ferritin of less than 50ng/ml. 25% of severe Hemophilia patients had a microcytic hypochromic peripheral smear. 4% were positive for

HBsAg; 2% for HIV and 2% for HCV. 18% had a history of receiving blood products for treatment.

CONCLUSION:

Most of the patients in the study group were severe haemophiliacs and majority of them had a low normal ferritin value with abnormal peripheral smear. The weight bearing joints were predominantly involved and the age at diagnosis was roughly around the same time as the infants begin to crawl. Blood borne infections were still common among the group. Only a very small percentage were aware of their mother’s carrier state.

KEY WORDS

FVIII – Factor VIII

FIX – Factor IX

FVII – Factor VII

Haemophilia A

Haemophilia B

Inhibitors

Arthropathy

X linked disorder

INTRODUCTION

Haemophilia a group of related bleeding disorders that are inherited. Inherited bleeding disorders include abnormalities of coagulation factors and platelet function; the most common of which is von Willebrand disease. However, when the term "haemophilia" is used, it most often refers to Haemophilia A and Haemophilia B. Our country has the second highest burden of haemophilia patients in the world. The pathophysiology of Haemophilia A and Haemophilia B is based on the insufficient generation of thrombin by the factor IXa/factor VIIIa complex through the intrinsic pathway of the coagulation cascade[1].

Bleeding may occur anywhere in patients with haemophilia. The most common sites are into joints and muscles and from the gastrointestinal tract. Approximately 80 percent of haemorrhage occurs in the joints; the ankles are most commonly affected in children, and the knees, elbows, and ankles in adolescents and adult. Spontaneous hemarthroses are characteristic of severe disease.

Haemophilia has an incidence of about 1 in 10000 in our country. Patients present with spontaneous bleeds or post traumatic bleeds depending on the severity of the disease. This group of patients are prone to develop chronic disability if under treated or untreated. Incidence of premature death is also high in this group of patients.

Iron deficiency might add on to the morbidity in Haemophiliacs. The iron deficiency can be due to the presence of occult blood loss in the urine and stools or due to the deposition of iron in the synovial membrane during repeated bleeding episodes[2]. Identifying iron deficiency and treating it in these patients might help to improve their quality of living.

Discovery of the molecular structure of both factor VIII and factor IX has recently allowed the development of genetically engineered products, prepared using recombinant DNA technology. In the long term, Preparations presently prepared form human plasma may be superseded by recombinant clotting factor concentrates (a DNA).

Nearly one third of cases of haemophilia occur with no preceding family history, possibly from new genetic mutation. When recorded family history is available, efforts should be made to identify female carriers. Identification depends on family history, measurement of clotting profile and DNA analysis.

AIMS AND OBJECTIVES OF THE STUDY

- Detailed Clinico haematological evaluation in patients with Haemophilia

- To identify serum ferritin levels in Haemophilia patients and to assess iron deficiency.

REVIEW OF LITERATURE

OVERVIEW

Haemophilia A is an X chromosome-linked hereditary disorder caused by defective synthesis or by synthesis of dysfunctional factor VIII molecules. Haemophilia A is less common than von Willebrand disease (vWD), but it is more common than other inherited clotting factor abnormalities. The clinical features of Haemophilia A and Haemophilia B are indistinguishable. Both are sex linked disorders.

They can present as mild moderate or severe forms. In the severe forms of both types of haemophilia spontaneous hemarthroses is common and can lead to chronic crippling hemarthropathy if not treated early or adequately. Deficiency of these factors leads to ineffective haemostasis due to ineffective thrombin formation. Highly purified concentrates and recombinant preparations are available for treatment and are considered safe and effective. The major complication is the development of antibody inhibitors against Factor VIII or IX but it is more common with Factor VIII.

HISTORY

The existence of a sex linked bleeding disorder was recognised in the 5th century in the Talmund. It was then referred to as "haemorrhaphilia" (love of bleeding) in 1828[1]. The disease was recognised when a rabbi identified that sons of haemophilia gene carriers were at risk of bleeding following circumcision. In

the 19th century several authors recognised the link between bleeding episodes to the delayed blood coagulation. Morawitz developed the classical theory of coagulation that involved 2 reactions –

1. Conversion of prothrombin to thrombin by a tissue substance that he named as thrombokinase

2. Conversion of fibrinogen to fibrin by thrombin[3].

In 1911, Addis contributed to the history of haemophilia by recognising that the defect is correctable with a small amount of normal plasma but he incorrectly attributed it to the deficiency of prothrombin. With the advent of better protein purification techniques in the 1930s and 1940s, the components of thrombokinase were identified.

Brinkhouse was the first to identify that the basic defect was a delayed conversion of prothrombin to thrombin. He corrected it by adding a small fraction of plasma containing antihaemophilic factor which was later named as factor VIII.

Until 1952 the presence of Haemophilia B was unnoticed. In 1947 Pavlovsky found that when he transfused blood from one Haemophilia patient into another patient, the clotting abnormality got corrected. But then he wasn't aware that he was dealing with two different types of haemophilia. In 1952, Aggeler and his co-workers described a patient deficient in "plasma

thromboplastin component," a blood clotting factor different from factor VIII. This factor was later named as Factor IX.

EPIDEMIOLOGY

International incidence of Haemophilia A is found to be 1 in 5000 to 7000 live male births. It occurs in all ethnic groups[3].

Severe Haemophilia - Factor VIII <1%

Moderate Haemophilia– Factor VIII 1 to 5 %

Mild Haemophilia – Factor VIII 6-30%

50 to 60% of patients have severe haemophilia, 25 to 30% have moderate haemophilia and 15 to 20% have mild haemophilia.

The annual incidence of Haemophilia A has been estimated at approximately 1 : 5000 male births . The incidence of Haemophilia B is estimated at approximately 1 : 30,000 male birth.[3]

The incidence of Haemophilia in India is about 1 in 10000 live male births. India is the second largest harbour of haemophilia patients. With the present records available number of Haemophilia patients in India is 11586 while the estimated prevalence is about 50000[4].

Prevalence indicates the number of patients with haemophilia alive at a given point of time. Some studies suggest a lower prevalence in the Chinese population and a higher prevalence among Caucasians[3].

Prevalence varies with age and the disease is maximum seen in the second and third decade of life.

Haemophilia B occurs 1 in every 25000 to 30000 male births. As with Haemophilia A, Haemophilia B also occurs in all ethnic groups and geographic distributions.

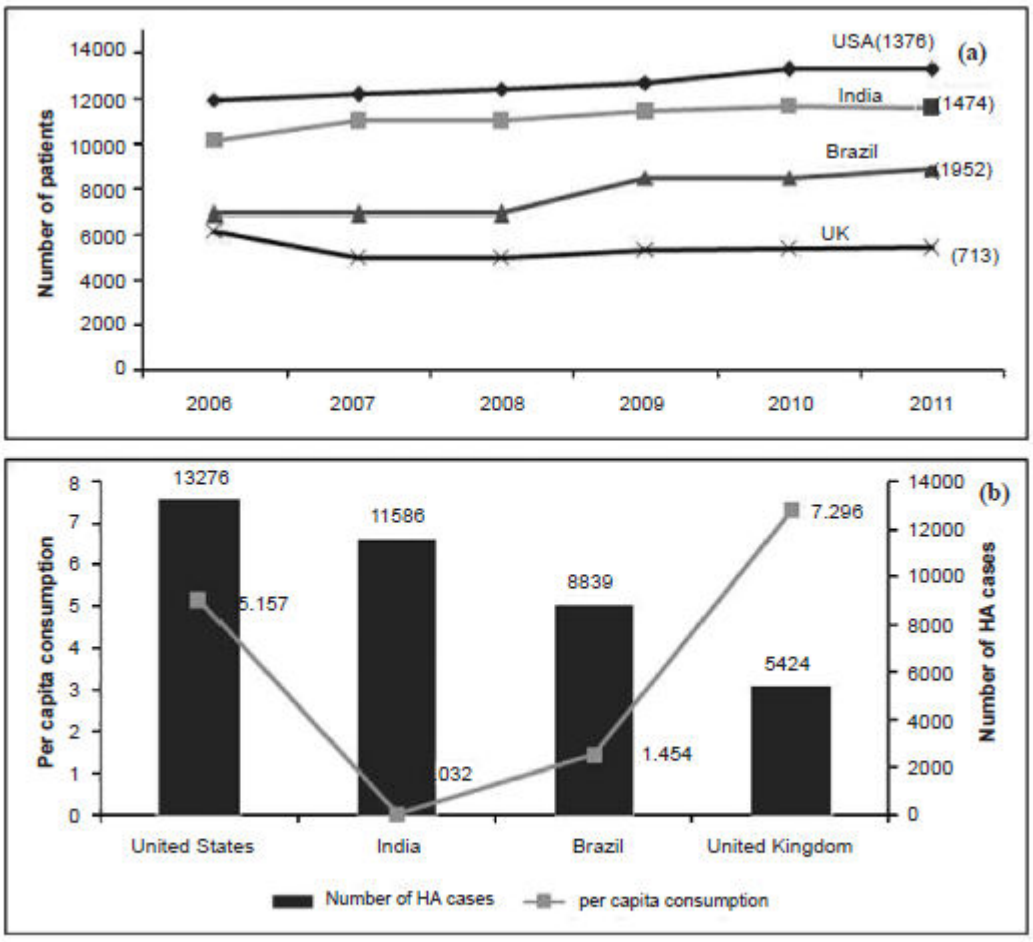


Fig. 5. (a) Haemophilia A trends in four high burden countries. Numbers in parenthesis show the number of patients reported in the last five years by each country (*Source*: Refs 29, 48, 49, 53-55. **(b)** Per capita clotting factor VIII consumption (Y axis) and number of haemophilia patients reported in 2011 for the four high burden countries (*Source*: Ref. 29).

Figure 1 Hemophilia trends in high burden countries

The per capita consumption of factor in India which has the second highest number of Haemophilia patient list, is only 0.032 as compared to The United States of America which has a factor consumption of 5.17.

EPIDEMIOLOGY IN INDIA:

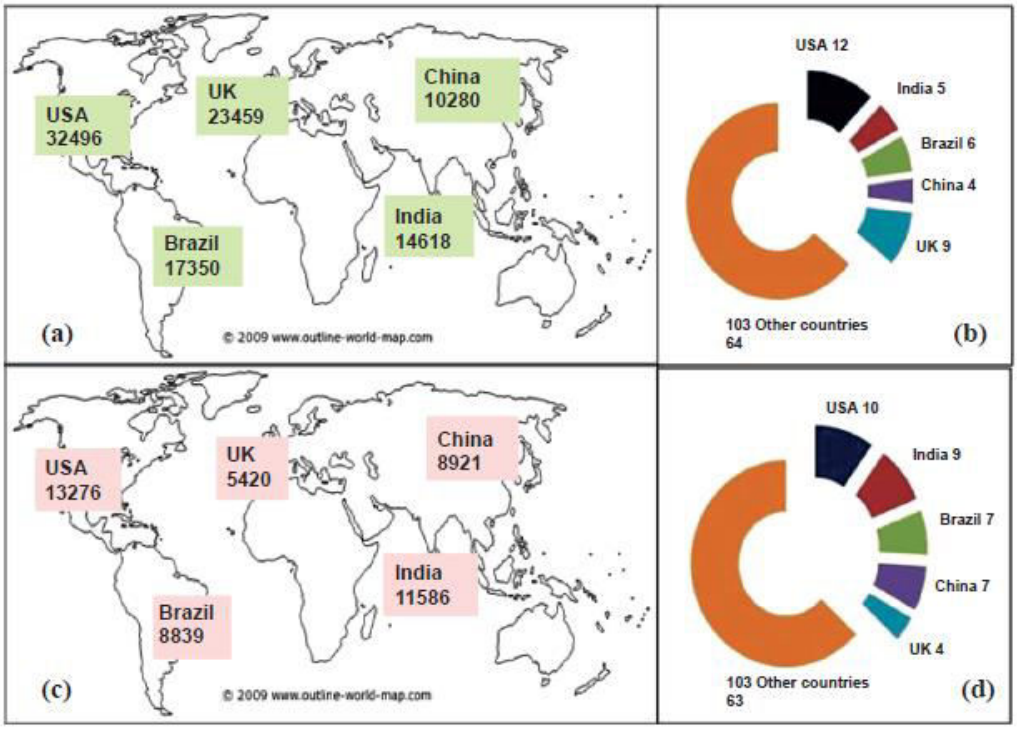


Fig. 3. Global distribution of total reported cases of bleeding disorders **(a)** and haemophilia A **(c)** in five countries reporting the highest number of patients. **(b)** and **(d)** show that nearly 5 and 9% of global patients with bleeding disorders and haemophilia A are from India. (*Source*: Authors' calculation based on data from Ref. 29).

Figure 2 Epidemiology

FUTURE PLANS IN INDIA

India lacks a proper surveillance system. With the data obtained from other developed countries that have an established surveillance system, the magnitude of cases in our country can be estimated. India could have around 70,000 patients as estimated from the prevalence data of USA. This large case

load might be due to the improving medical services or due to the increased life span of these patients.

With the expected high case load, the need for a national programme for Haemophilia must be emphasized. The problem of equity should be kept in mind. People from the lower socio economic status and rural areas must be made accessible to the factors. Compulsory Hepatitis B vaccination must be emphasized. Routine screening for HIV and HCV should be done for persons with Haemophilia.

The data on epidemiology and the cost incurred in treating a patient is lacking in our country. However, data regarding the genetic basis of the disease and the prenatal diagnosis is available. Pre natal diagnosis is carried out in a few premier institutes within the country.

Maintaining a Haemophilia registry is important in order to obtain information regarding the disease burden and trends in our country.

ETIOLOGY

Haemophilia A is a heterogeneous disorder resulting from the reduced levels of functional factor VIII in the peripheral circulation. This can be due to either decreased levels of factor VIII or decreased functionality of factor VIII.

Factor VIII must be activated by thrombin for it to be an effective cofactor of factor IXa. Factor IXa's capacity to activate Factor X is exponentially increased in the presence of activated factor VIII - FVIIIa. Thus the clinical manifestations of Haemophilia A and B are not very much different from each other. Together Factor IXa and VIIIa (X-ase) activate factor X which is necessary for effective thrombin formation. In the absence of either factors clot formation is delayed and the clot thus formed is easily friable, dislodged and subjected to fibrinolysis which leads to excessive bleeding.

GENETICS

Haemophilia A and B are X linked recessive disorders. About 30% of Haemophilia A mutations can arise de-novo. The gene for Factor VIII is large with 26 exons and 25 intervening introns. This makes identification of the mutation difficult.

All the sons of affected haemophiliac males are normal while all the daughters are obligatory carriers. Sons of carriers have 50% chance of being affected and the daughters of the carriers have a 50% chance of being carriers themselves. In female carriers, because of lyonization there might be

preferential expression of the defective haemophilic allele. Such females who have Haemophilia are called “lyonized carriers”. Other mechanisms for female Haemophilia include homozygosity for the defective Factor VIII allele and hemizygosity for the defective gene. In female haemophiliacs, karyotyping is necessary to rule out Turner’s syndrome and testicular feminization which has been associated with Haemophilia A. No single mutation can cause Haemophilia. Hundreds of deletions, point mutations and inversions have been identified.

Analysis of Factor IX mutations show that it occurs due to endogenous processes – deamination of CpG dinucleotides rather than from environmental effects. As with Haemophilia A, no single gene has been attributed to the development of Haemophilia B.

FACTOR VIII gene and HAEMOPHILIA:

Factor VIII gene contains 186 kb with 26 exons, and produces an mRNA transcript of 9kb which is translated into 2351 amino acid polypeptide. The mature protein is divided into homologous domains named A1, A2, B, A3, C1, C3.The most common defect is inversion of 500-600 kb region that results in disruption of F8 region of intron 22. Additoinal inversion of Intron 1 of Factor VIII contributes to 5% of severe Haemophilia. Point mutations involving CpG dinucleotide are more common.

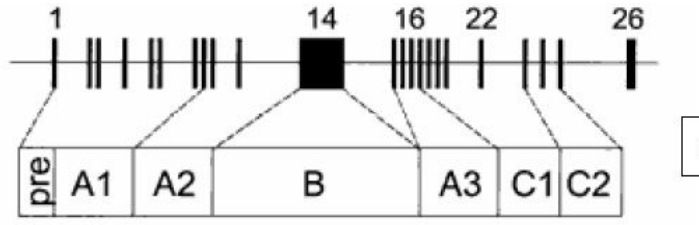


Fig.5:FVIII gene exons

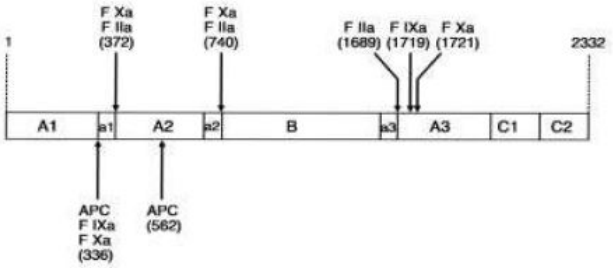


Fig.6:Factor VIII peptide

Figure 3 Factor VIII gene

FACTOR IX GENE AND HEMOPHILIA B:

The gene for factor IX is located on the long arm of chromosome X. It is much smaller than the gene for factor VIII measuring only around 33kb.

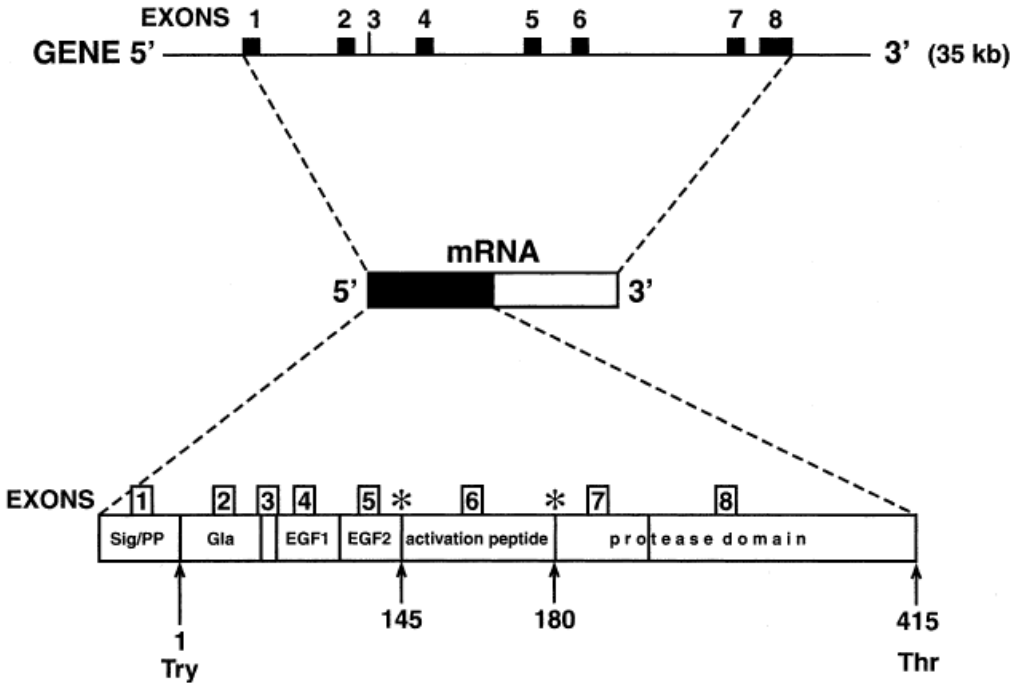
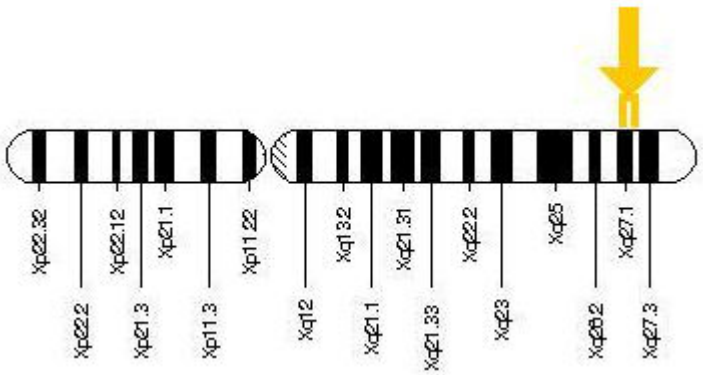


Figure 4 Factor IX gene

It has been extensively studied when compared to Factor VIII. Eight hundred ninety-six distinct mutations in the factor IX gene have been reported in the factor IX database, including more than 500 distinct amino acid substitutions and 41 complete gene deletions[1]. Majority of these mutations involve the CpG dinucleotide that affects critical arginine molecule, resulting in a dysfunctional protein. Haemophilia B Leiden phenotype involves mutation in the 5' promoter region of the gene. This disorder is characterised by very low levels and activity of Factor IX at birth, that gradually increases to 60% after puberty probably due to the effects of androgen.

F9 is the gene's official symbol. The *F9* gene is located on the long (q) arm of the X chromosome between positions 27.1 and 27.2. More precisely, the *F9* gene is located from base pair 139,530,732 to base pair 139,565,696 on the X chromosome. Other names are Christmas factor, Plasma thromboplastin component, HEMB,FA_9HUMAN and a few others.



MUTATIONS IN THE PROMOTER EGION OF FACTOR IX

Nucleotide Substitution	Nucleotide Change	Factor IX Percent Activity	Factor IX Percent Antigen	Comments
−21	T→G	<1–70	—	Disruption of HNF-4 binding site; factor IX activity increases after puberty
−20	T→A	<1–60	<1–60	a
−20	T→C	9	—	a
−6	G→A	13–70	—	a
−5	A→T	3	—	a
6	T→A	<2–20	—	a
8	T→C	1–32	—	C/EBP binding site: factor IX clotting activity increases after puberty
13	A→G	<1–60	<1–60	b
13	delete 1	<1–60	<1–60	b

ACTIVATION OF FACTOR IX:

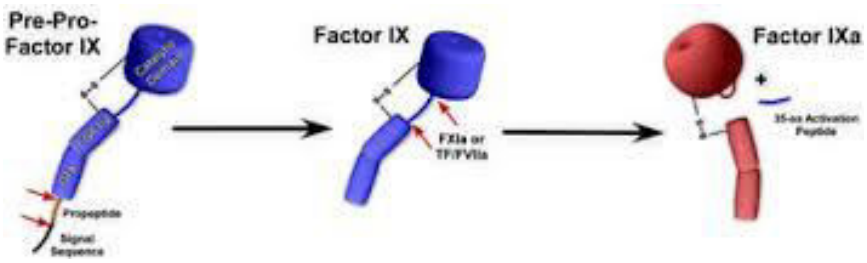


Figure 5 Activation of factor IX

FACTOR IX GENE:

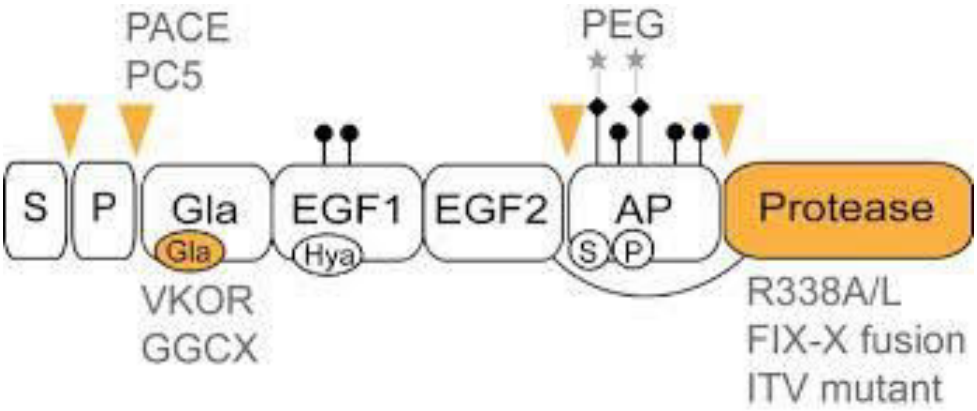


Figure 6 Factor IX gene

GENETICS IN HEMOPHILIA:

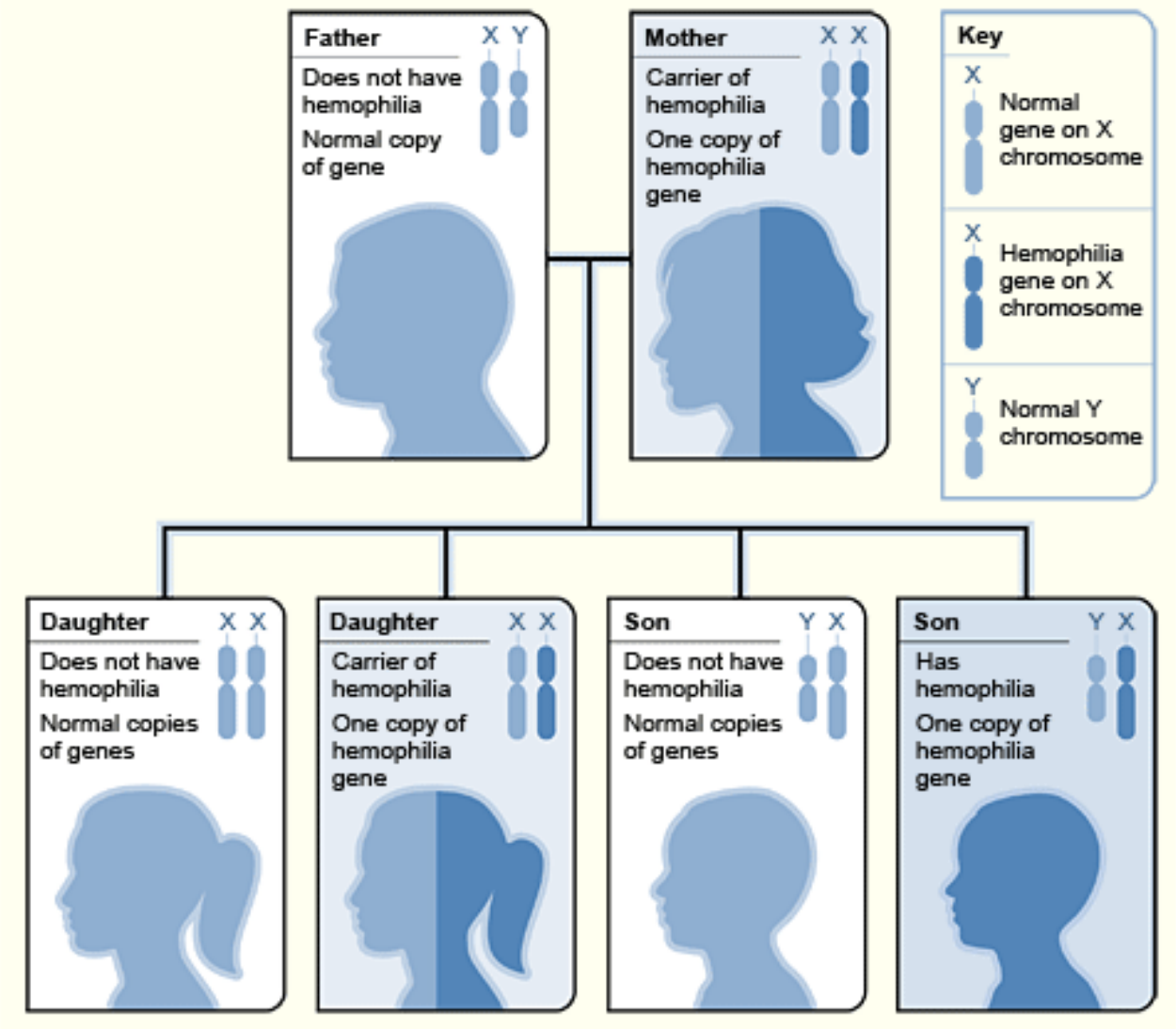


Figure 7 Pedigree

When a carrier woman marries a normal man, there is a 50% chance of the girl children to be carriers and 50% chance of her son to be a Haemophilic.

CLOTTING CASCADE :

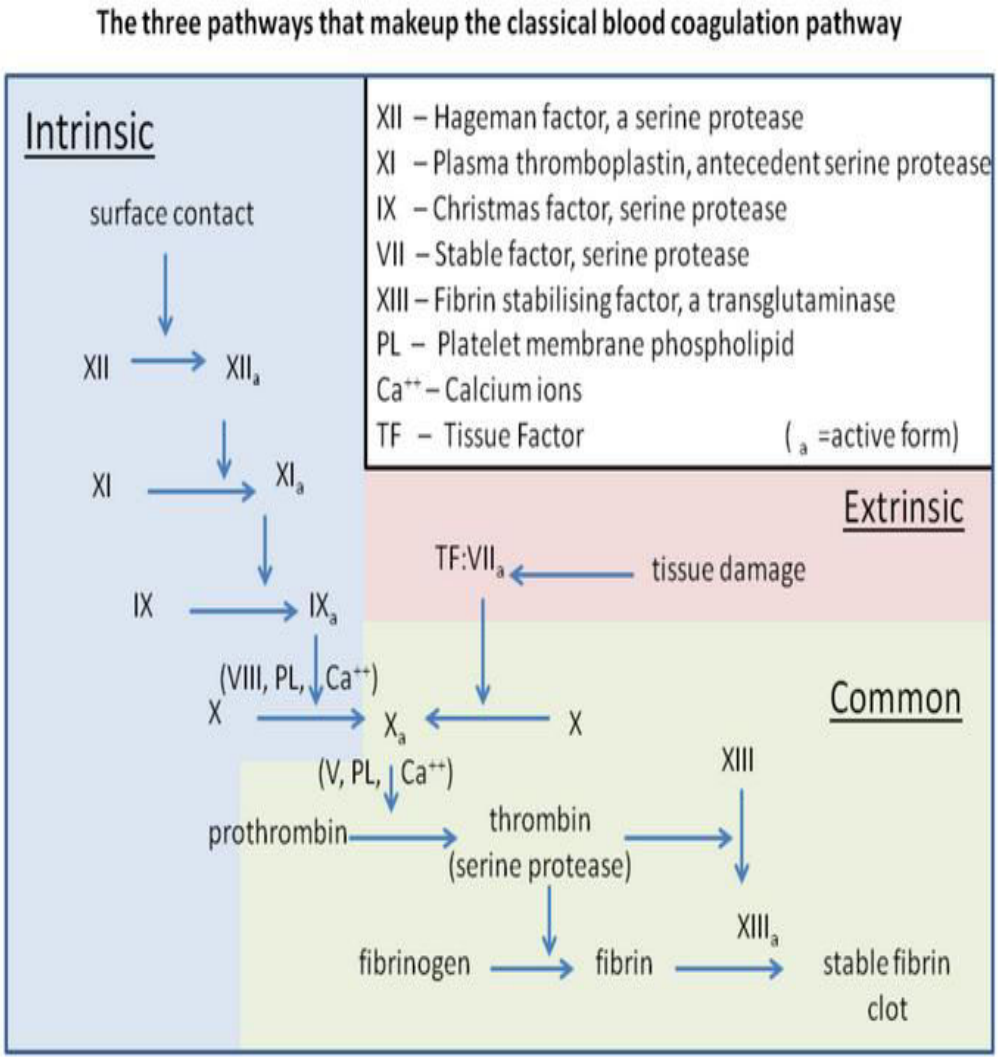


Figure 8 Clotting cascade

CLOTTING CASCADE:

1. VASCULAR CONSTRICTION
2. PLATELET ACTIVATION
3. EXTRINSIC PATHWAY
4. INTRINSIC PATHWAY
5. FINAL COMMON PATHWAY
6. COFACTORS
7. REGULATORS
8. FIBRINOLYSIS

VASCULAR CONSTRICTION:

After a trauma, the blood vessel immediately constricts causing reduced blood leakage. This occurs due to local myogenic reflex, local autocoid factors released by the vessel wall and the platelets and neurogenic reflex. The neurogenic reflex originates from the pain nerve endings. Of the above mentioned factors, the myogenic reflex is the most powerful. Of the autocoids, the most important vasoconstrictor is the thromboxane A2. The more the injury to the vessel, greater is the spasm. The spasm might last from few hours to days during which time the coagulation cascade is activated and the process continues.

PLATELET ACTIVATION:

If the cut vessel is very small, it can be sealed by the platelet pug itself. Many such small leaks keep happening throughout the day.

PLATELET PHYSIOLOGY:

Platelets or thrombocytes, formed from the megakaryocytes in the bone marrow, are small discs measuring about 1 to 4 micrometers in diameter. The normal concentration of platelets is around 1,50,000 to 3,00,000 per litre.

The cytoplasm of platelets contain,

- Contractile proteins like actin, myosin and thrombasthenin
- Golgi bodies and Endoplasmic reticulum that store large quantities of calcium
- Mitochondria, that synthesise ATP and ADP
- Enzyme system that synthesizes prostaglandins that act as local hormones and play major role in haemostasis.
- Fibrin stabilizing factor
- A growth factor that causes vascular endothelial cells, smooth muscle cells and fibroblasts to grow.

The platelets' cell membrane has glycoprotein that repulses attachment to the vessel wall but gets attached to injured endothelium and exposed collagen. In addition platelets contain phospholipids that activate various stages of blood coagulation process. The half life of platelets is 8 to 12 days.

When the platelets come in contact with collagen they change their characteristics. They tend to swell up and the contractile process within the platelets release the contents of the granules. These make the platelets even more sticky and they tend to bind avidly to each other and to the exposed collagen and von willebrand factor. It also releases ADP which forms Thromboxane A2. This further makes the platelets sticky. A platelet plug is formed which is initially loose and then tends to become closely packed. Small leaks are closed by platelet plugs themselves. This is the reason why patients with thrombocytopenia develop multiple small bleeding spots under their skin throughout the day which never happens in a normal individual.

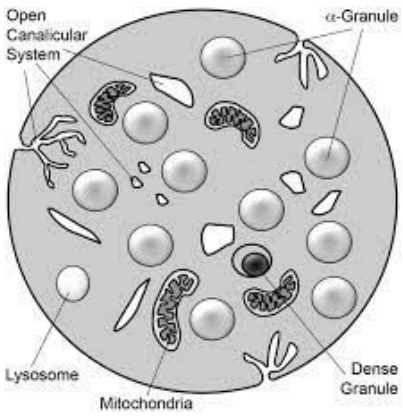


Figure 9 Platelet morphology

The coagulation process is initiated by three factors,

- Damage to bleed vessel
- Damage to the blood components
- Exposure of blood to damaged endothelial cells or the underlying collage

In each instance, it leads to the formation of the prothrombin activator which converts prothrombin to thrombin and the coagulation continues.

The prothrombin activator can be formed by two processes,

- EXTRINSIC PATHWAY – Begins with the trauma to the vessel wall
- INTRINSIC PATHWAY – Begins in the blood itself

EXTRINSIC PATHWAY:

This is also known as tissue factor pathway. This begins with the exposure of blood to the damaged extra vascular tissue or traumatised vessel wall.

STEPS:

1. Release of TISSUE FACTOR: Damaged vessel releases several tissue factors or tissue thromboplastin. They are composed of phospholipids and act as proteolytic enzyme.

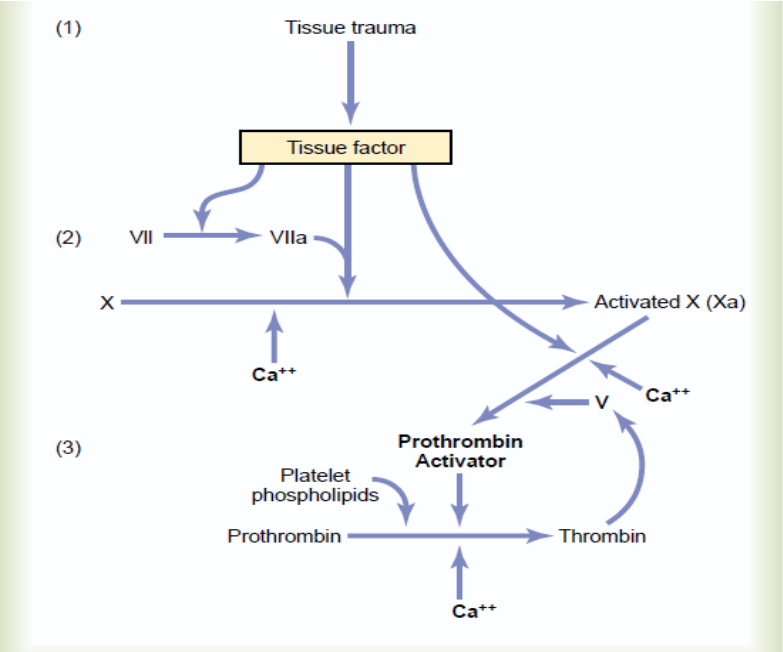


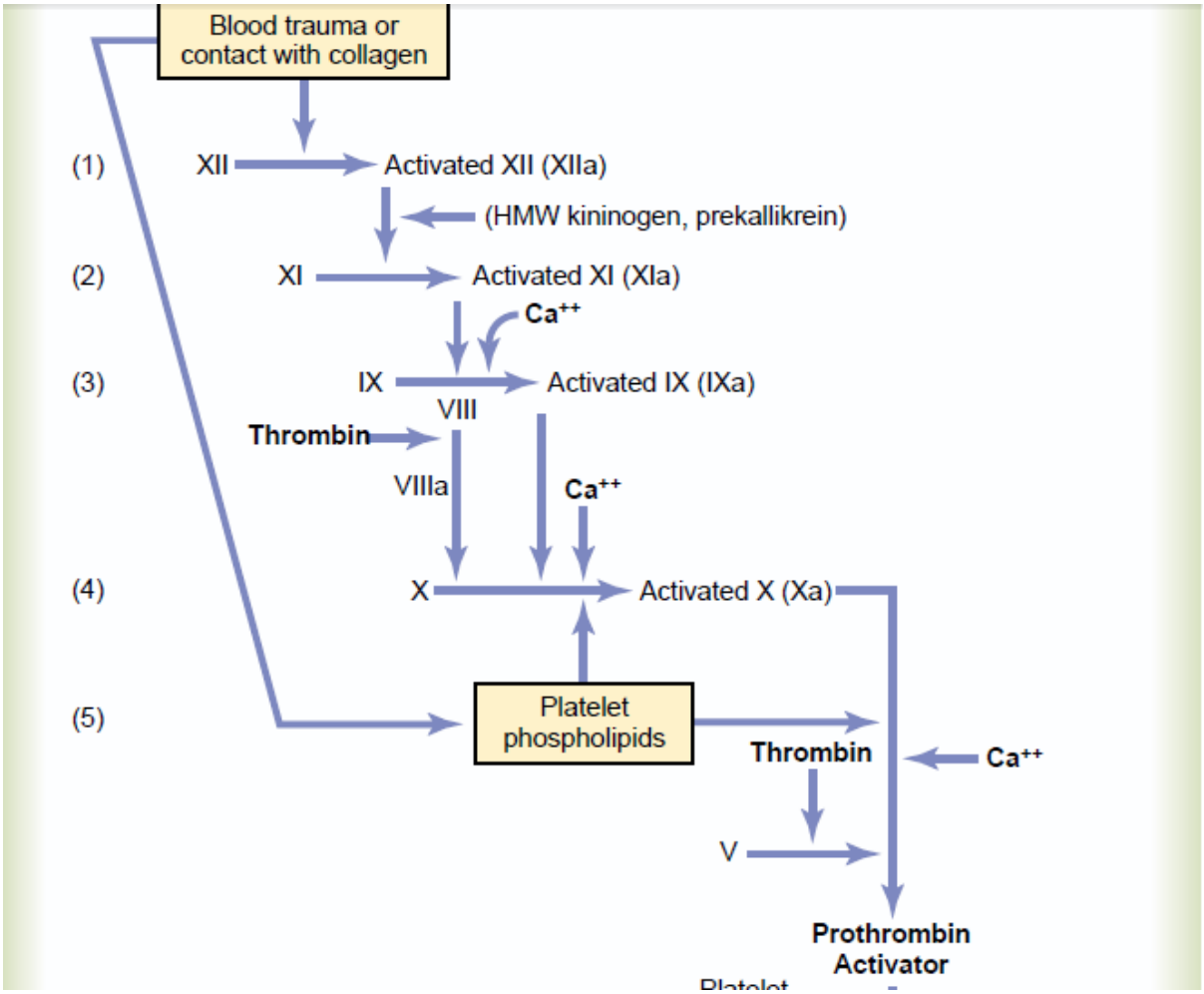
Figure 10 Extrinsic pathway

2. FACTOR VII AND TISSUE FACTOR: The lipoprotein tissue factor forms complexes with Factor VII and in the presence of Calcium catalyzes the activation of Factor X to Xa.
3. Factor Xa combines with Facto V and in the presence of calcium becomes the PROTHROMBIN ACTIVATOR.
4. Prothrombin activator complex in the presence of calcium converts prothrombin to thrombin and the coagulation continues.

In the prothrombin activator complex, Factor X is the actual protease and Factor V accelerates the protease activity. The formed thrombin further activates Factor V which becomes an additional accelerator of prothrombin activator thereby becoming a positive feedback mechanism.

INTRINSIC PATHWAY:

The second pathway for initiating coagulation is the intrinsic pathway which gets activate with damage to the blood components.



1. Trauma to blood causes activation of Factor XII. When Factor XII comes in contact with wet able surfaces like collagen it gets activated to a protease – Factor XIIa. Simultaneously, platelets release platelet factor 3 which in turn contributes to the coagulation cascade.
2. Factor XIIa activates Factor XI to XIa. This reaction needs High molecular weight kininogen and prekallikrein.
3. The activated Factor XI then enzymatically cleaves Factor IX to form the activated Factor IXa.
4. The activated Factor IXa, along with activated factor VIIIa, Platelet factor 3 and platelet phospholipids end in activating Factor X to Xa. Factor VIII is also known as the antihaemophlic factor.
5. This step is the same as in intrinsic pathway. The activated Factor X combines with Factor V to form the prothrombin activator complex.
6. The prothrombin activator causes conversion of prothrombin too thrombin thereby setting the coagulation cascade into motion.

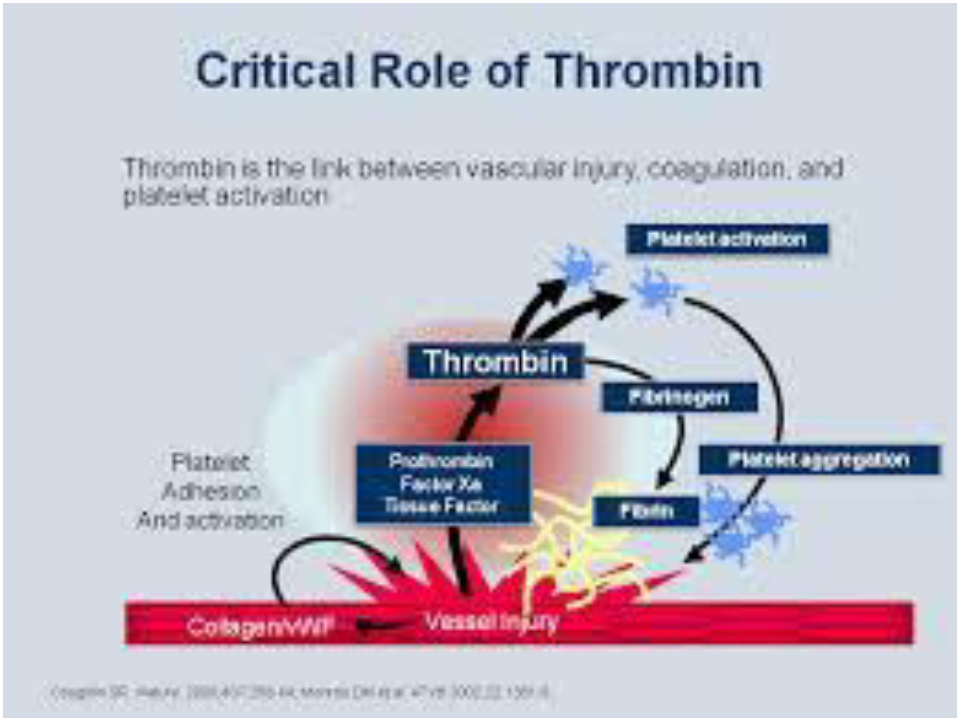
CONVERSION OF PROTHROMIN TO THROMBIN:

After the formation of prothrombin activator by either the intrinsic or extrinsic pathway, it causes conversion of prothrombin to thrombin in the presence of ionic calcium. Thrombin causes polymerization of fibrin molecules within 10 to 15 seconds. So, the rate limiting step in coagulation process is the formation of the prothrombin activator and not the steps that happen beyond it.

Platelets also play a role in the conversion of prothrombin to thrombin as most of the prothrombin binds to the prothrombin receptors on the surface of the platelets.

PROTHROMBIN AND THROMBIN:

Prothrombin is formed by the liver and it needs Vitamin K for formation. The normal plasma concentration is about 15mg/dl. It is constantly being used up in the body for coagulation process. In the presence of liver failure or vitamin K deficiency, prothrombin production is grossly reduced and can result in coagulopathy.



Thrombin is the link between vascular injury, coagulation and platelet activation.

CONVERSION OF FIBRINOGEN TO FIBRIN AND CLOT FORMATION:

FIBRINOGEN:

Fibrinogen is a high molecular protein, (MW=3,40,000) with a plasma concentration of 100 to 700mg/dl. It is produced in the liver and so liver disease can result in reduced plasma levels of fibrinogen. Because of its high molecular weight, little, leaks outside the blood vessels. In case of trauma, it leaks out and can cause clotting of tissue fluids just like plasma and blood.

CLOT RETRACTION:

Within a few minutes after clot formation, it begins to contract expressing the serum. In this way serum differs from plasma because it cannot clot as the clotting factors have been depleted. The platelet themselves contribute to the process of clot retraction. Clot retraction is affected in case of thrombocytopenia. The platelets bring the fibrin molecules together. The contractile process within the platelets get activated and this is involved in clot retraction. It also releases procoagulant factors. The contraction is activated by thrombin and accelerated by the calcium released from the endoplasmic reticulum and golgi apparatus within the platelets.

As the clot retracts, the edges of the broken blood vessels are pulled together there by contributing further to complete haemostasis.

COFACTORS:

Substances required for the proper functioning of the coagulation cascade:

CALCIUM

VITAMIN K

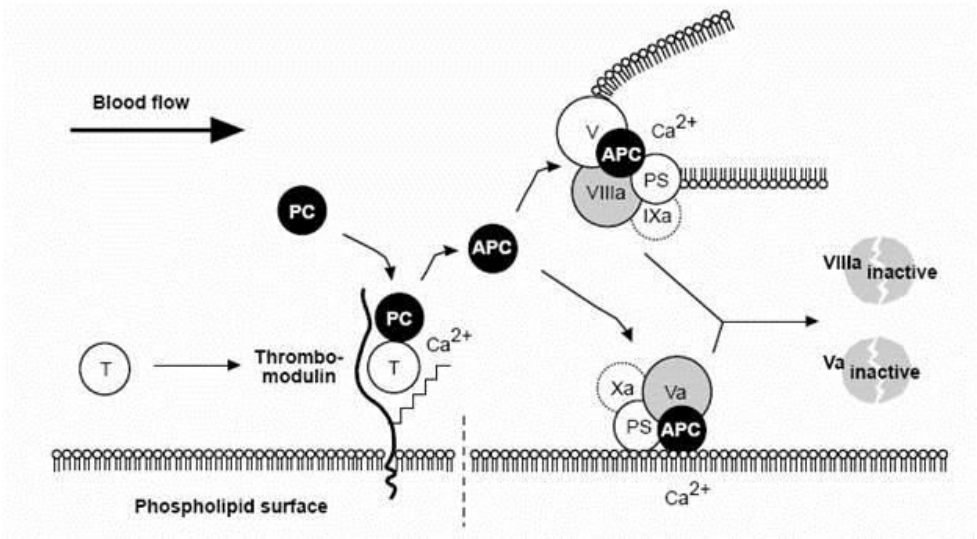
- Except for the first two steps of the intrinsic pathway, Calcium ions are needed for the activation and propagation of the entire coagulation cascade. In live, the levels of ionised calcium rarely become low enough to affect the coagulation. But after sampling, blood can be maintained in the liquid state by adding citrate, that binds with the ionised calcium or oxalate, that precipitates the calcium.
- Vitamin K is essential for the gamma carboxylation of Factor II, VII, IX and X. This process is essential for the factors to bind to phospholipids and there by participate in the coagulation cascade. In the absence of vitamin K, either due to deficiency or liver disease, PIVKAs (Proteins formed in the absence of Vitamin K) are formed. These are defective proteins and do not participate effectively in the coagulation cascade.

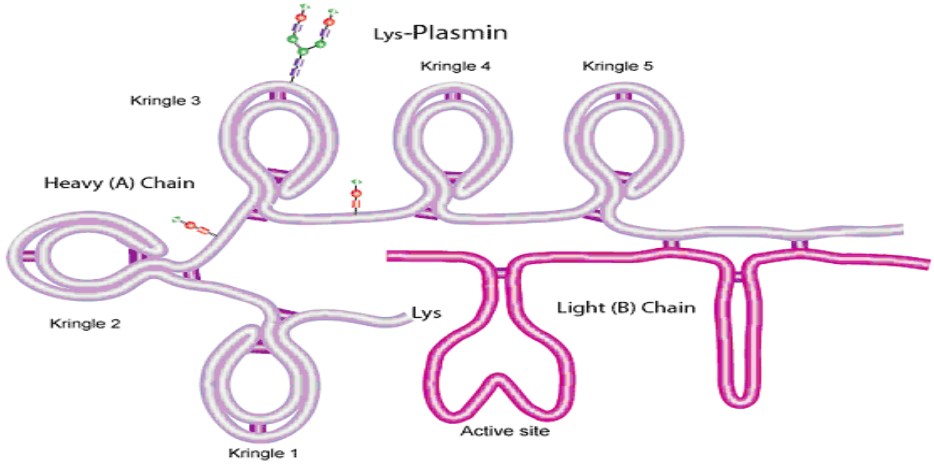
REGULATORS:

There are four mechanisms that keep platelet activation and the coagulation cascade in check. Abnormalities can lead to an increased tendency toward thrombosis:

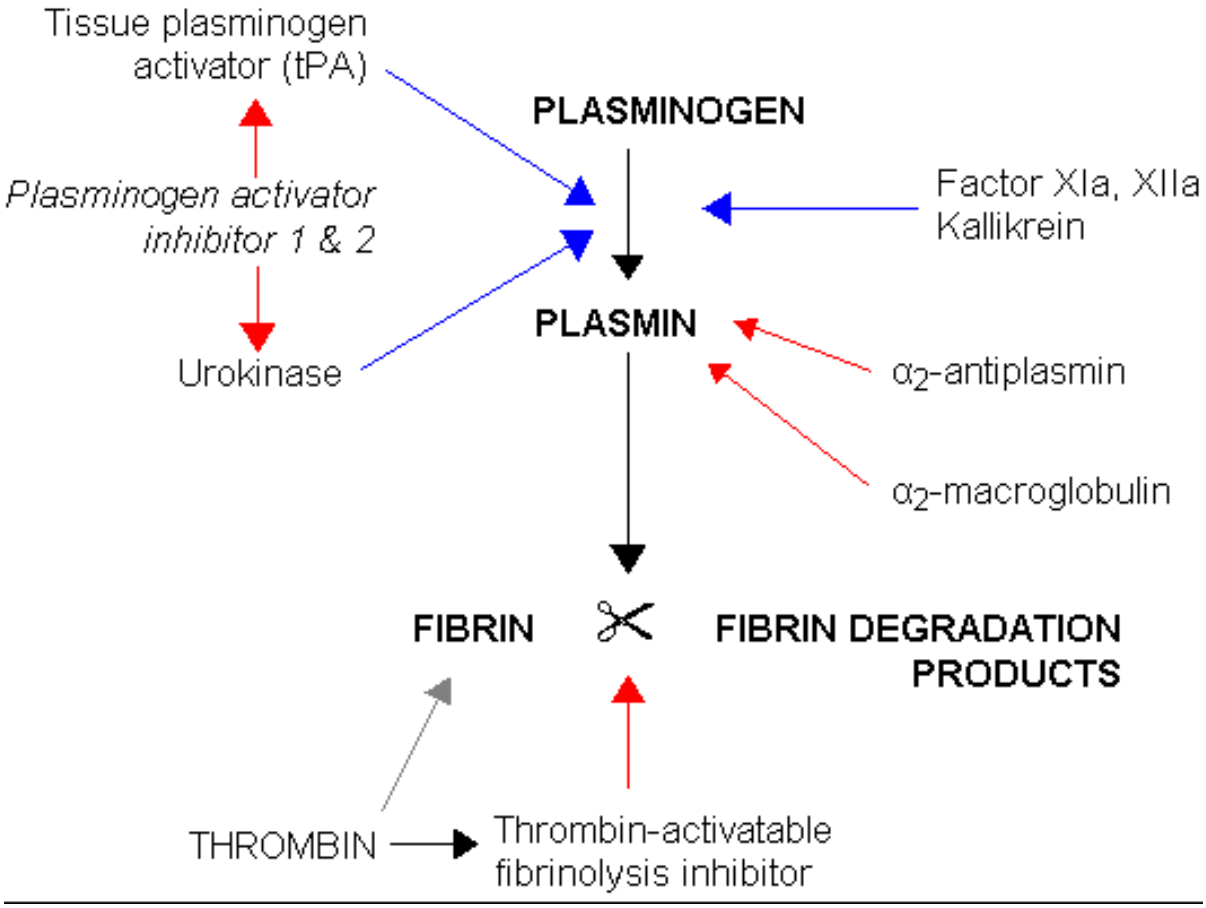
- ***Protein C*** is a trypsin like peptide molecule. The gene for Protein C is on chromosome 2. It undergoes a lot of post translational modification at the amino terminal end. Thrombin activates protein C into aPC. Activated protein C inhibits factor Va and VIIIa. This causes termination of the role of factor VIIIa in the tenase complex and the role of factor Va in forming the prothrombin activator complex. The net effect on the coagulation cascade is inhibition of further fibrin and there by clot formation. Apart from the anti coagulant effect, it also has cytoprotective and anti-inflammatory properties by binding to the endothelial protein c receptor and activating PAR 1.
- ***Antithrombin*** is a serine protease that inhibits factor Xa and thrombin. The normal plasma concentration is 0.15 to 0.20 mg/ml. Antithrombin deficiency can cause thrombotic disorders. There are two types. Type 1, in which the level of the protein is reduced and Type 2 , in which the functional activity of the protein is reduced.
- Tissue factor pathway inhibitor (TFPI) limits the action of tissue factor (TF) and also inhibits excessive TF-mediated activation of Factor VII and Factor X.

- Plasmin is produced from the plasminogen, a zymogen produced in the liver. While in circulation, plasminogen adopts a closed conformation. After binding to clots it adopts an open conformation and can be activated to plasmin by a variety of factors, the most important of which is Tissue plasminogen activator. Plasmin deficiency, which is rare in humans, can cause thrombosis.





ROLE OF APC AND PLASMINOGEN:



Blue arrows – Stimulation

Red arrows - Inhibition

MUTATIONS IN HAEMOPHILIA :

	Missense[a]	Nonsense (Stop)	Splicing[b]	Small	Large	
1	14	1	4	2	—	0
2	5	1	5	6	—	2
3	24	0	3	4	—	0
4	24	5	2	1		1
5	11	1	7	3		1
6	6	0	3	4		2
7	32	4	0	6		0
8	21	4	1	8		1
9	22	3	1	5		1
10	10	1	0	4		0
11	27	1	3	1		1
12	20	5	2	0		1
13	23	3	2	4		1
14	37	39	3	63	—	31
15	13	1	4	2	—	0
16	18	4	2	5	—	0
17	24	3	1	5	—	4
18	26	4	1	3	—	3
19	14	4	5	3	—	1
20	5	1	0	0	—	1
21	5	4	0	0	—	1
22	16	5	5	3	—	1
23	25	1	4	6	—	0
24	10	3	3	3	—	2
25	11	2	1	4	—	2
26	19	3	0	7	—	0

PRENATAL DIAGNOSIS AND CARRIER DETECTION :

Prenatal diagnosis can be suggested to woman those who are related to obligate Haemophilia carriers and known Haemophiliacs. Germ line mutations have different implications when it occurs in the grandfather and the mother. Prenatal diagnosis can be done using cells obtained during amniocentesis done at 16 weeks of gestation. If the foetus is a female little is the concern as carrier females rarely have bleeding manifestations. In case of a male foetus, the diagnosis can be made by DNA analysis of the cells. Termination or continuation of the pregnancy to term is left to the decision of the parents.

DNA based methods are preferred for carrier detection. Studies identified the causative mutation in 90% of patients with mild and moderate haemophilia A but only 50 to 60% of patients with severe disease. Direct gene analysis for the inversion of intron 22 is recommended in all females suspected to be carriers.

The analysis is performed in the eleventh to twelfth week of gestation either by amniocentesis or chorionic villus sampling.

CLINICAL PRESENTATION

Clinical presentation of Haemophilia A and B are indistinguishable. Both are very similar in their presentation.

Severe Haemophilia refers to factor levels less than 1% of normal or less than 0.01u/ml. They present in early infancy with spontaneous haemorrhage. Frequent spontaneous hemarthroses and haemorrhage are common and they require frequent factor replacement.

Moderate haemophilia have factor levels between 1 to 5% of normal. They present with increased bleeding after a minor trauma and surgery. Spontaneous bleeds are rare and occasionally they present with spontaneous hemarthroses.

Mild haemophilia refers to factor levels of 6 to 30% of normal. These patients bleed secondary to surgery and rarely do they present with spontaneous hemarthroses.

Some of the severe Haemophiliacs do not manifest the severe bleeding and they have been identified to have the Factor V Leiden mutation.

The bleeding episodes in Haemophiliac patient is intermittent. Some do not bleed for weeks together. Death from bleeding occurs only in intracranial haemorrhage.

Most carriers have factor levels greater than 50% and do not have bleeding manifestations. Those with factor levels less than 50%, due to extremely imbalanced X chromosome mutation, have bleeding post trauma or surgery. Factor VIII levels should be checked in all Haemophilia carriers.

The most common presenting clinical symptom in moderate and severe haemophilia is intra articular and intra muscular bleeds. These symptoms tend to occur only after the child begins to ambulate. In fact, the most common *presenting* manifestations of Haemophilia A and B in one series of patients were soft-tissue bleeds in 41%, bleeding associated with intramuscular injections and surgery in 16%, and oral bleeding from tongue or lip biting in 11%,14% of which were severe enough to require transfusion of packed red blood cells[1].

PATIENTS WITH SEVERE DISEASE HAVING MILD MANIFESTATIONS – REASONS:

1. The Leyden phenotype of Haemophilia B is characterized by severe haemophilia in childhood that becomes mild after puberty. The mutation is at the nucleotide 20 promoter region and it disrupts the HNF-4 binding site but not the overlapping site for androgen binding. This might explain the recovery after puberty[5].
2. Coinheritance of Leiden V and other prothrombotic states occurs in a small percentage of Haemophiliacs and this might counteract the bleeding from decreased factor levels. Patients with such coinheritance experience fewer bleeds and also have a later onset of first bleed[5].

INTRAPARTUM COMPLICATIONS:

A study of the modes of delivery and peri-natal complications in affected male babies shows that the risk of intracranial bleed is less in normal delivery. (<3.8%)[3]. The risk of subgaleal and cephalic hematoma increases with vacuum delivery. Caesarean section does not eliminate the risk of intracranial haemorrhage. Forceps delivery and prolonged labour increase the risk of intracranial bleeds. However, the use of scalp electrodes and intrapartum blood sampling for affected neonates does not increase the risk of bleeds in the neonate[1].

Seizures are common during the acute intracranial bleed episode. Psychomotor complications and cerebral palsy can occur as long term complications. Most episodes of intracranial bleed in newborns occur in sporadic cases and with the use of vacuum apparatus. In a European study, involving 508 children born with Haemophilia A or B, intracranial bleeds occurred in 18 (3.5%) within the first 28 days of life[5].

Vaginal delivery with forceps or assisted vacuum must be avoided in woman who are known carriers of Haemophilia and are known to be pregnant with a male child.

CIRCUMCISION AND BLEEDING:

50% of undiagnosed haemophiliacs have excessive bleeding during circumcision that can be stopped with factor infusion. Such excess bleeding occurs in less than half of the affected infants. Thus, failure to bleed does not eliminate the presence of Haemophilia in that patient.

AGE OF BLEED ONSET:

Children with severe haemophilia become symptomatic within the first 2 yrs of life. In a study, the mean age of first bleed leading to the diagnosis of Haemophilia was at 0.9 yrs of age, in the absence of any prothrombotic factor coinheritance.

In the presence of prothrombotic factors, the mean age for diagnosis was late and was around 1.6 years of age[5]. However some patients do not bleed before the age of five. Joint bleeds are very common and early diagnosis would help to prevent the joints from hemarthropathy.

The age of diagnosis in mild and moderate Haemophilia is later than that for severe haemophilia. In a study of 140 boys from Sweden, the mean age of diagnosis of Moderate and severe disease was found to be 22 and 9 months respectively[5].

Mild haemophilia without family history can go undetected for a very long period of time, as about one third of patients have very few bleeding episodes.

- HEMARTHROSES
- HEMATOMAS
- PSEUDO TUMORS
- HEMATURIA
- NEUROLOGICAL COMPLICATIONS
- MUCOUS MEMBRANE BLEEDS
- POST SURGICAL BLEEDS

HEMARTHROSES:

Hemarthroses is the most common bleeding manifestation in severe haemophiliacs contributing to about 75% of the bleeding episodes.

Anatomy of the synovium favours bleeding, It has numerous cells and abundant capillaries that lie beneath the synovial layer. These capillaries are susceptible to damage during mechanical trauma associated with the daily use of joints. The joints involved in decreasing order of frequency are knee, elbow, ankle, shoulder, elbow and hip. Hinge joints are more commonly affected than ball and socket joints.

Hemarthroses might produce an aura of discomfort that gradually progresses to cause joint enlargement and excruciating pain. The joint becomes swollen, warm and tender with decreased range of movement. Patient might have a mild

fever during the bleed. However, sustained fever indicates an infected joint. When bleeding stops, the blood is reabsorbed in a couple of days. If the bleed is treated early and the joint is not affected chronically, pain subsides in 6 to 8 hours and disappears in 12 to 18 hours. However, repeated bleeding into the joint causes articular destruction and leads to haemophilic arthropathy. Once chronically affected it may be difficult to distinguish the pain of degenerative arthritis from the pain of bleeding.

The synovium becomes thickened and folded leading to repeated bleeds in the same joint causing the so called target joint. The joints most often involved are the weight bearing joints, knee and ankle. Bleeding into a joint with thickened synovium causes less pain than bleeding into a normal synovium.

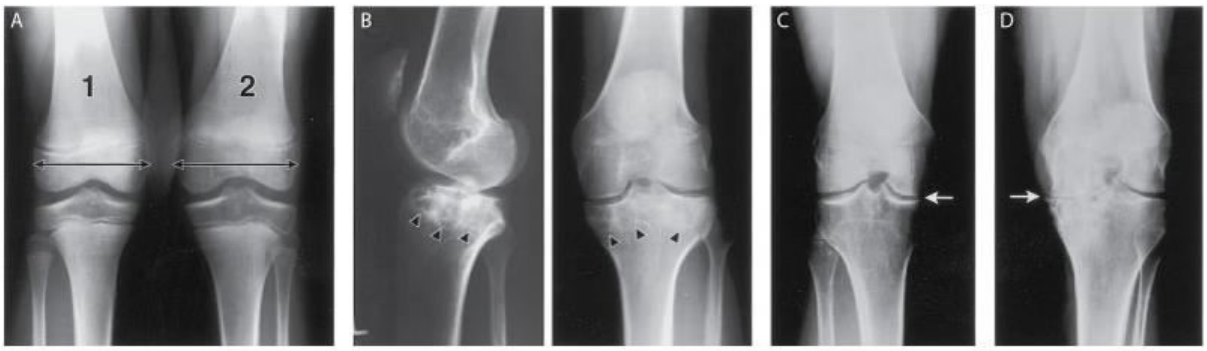
In the presence of fever, leukocytosis and other systemic manifestations the probability of an infected hemarthroses should be considered. Rapid diagnosis is a must as infection in such joint progresses rapidly causing loss of joint space and architecture. The joint should be aspirated under strict aseptic techniques and factor replacement should be given. Chronic haemophilic arthropathy is painful with weight bearing but the pain subsides or disappears once the joints become ankylosed. Muscle atrophy around the joint can lead to increased inability and increased bleeding from the loss of cushioning effect provided by the muscles. Patients with factor VIII or IX deficiency with levels greater than 20% of normal rarely develop haemophilic arthropathy even if they have experienced previous bleeds[1].

Socio economic status and other health effects have an influence on the morbidity of haemophilic arthropathy. Obesity is common among haemophiliacs because of repeated bleeds and sedentary lifestyle. This further has an effect on the range of movements of the joints.

Haemophilic arthropathy – Chronic effects of repeated knee bleeds

Source: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology*, 7th Edition: http://www.accessmedicine.com

Radiographic stages of haemophilic joint :



Stage 0 – Normal joint

Stage 1 – fluid in the joint

Stage 2 – Osteoporosis and epiphyseal overgrowth – Fig A

Stage 3 – Subchondral bone cysts – Fig B (arrowheads)

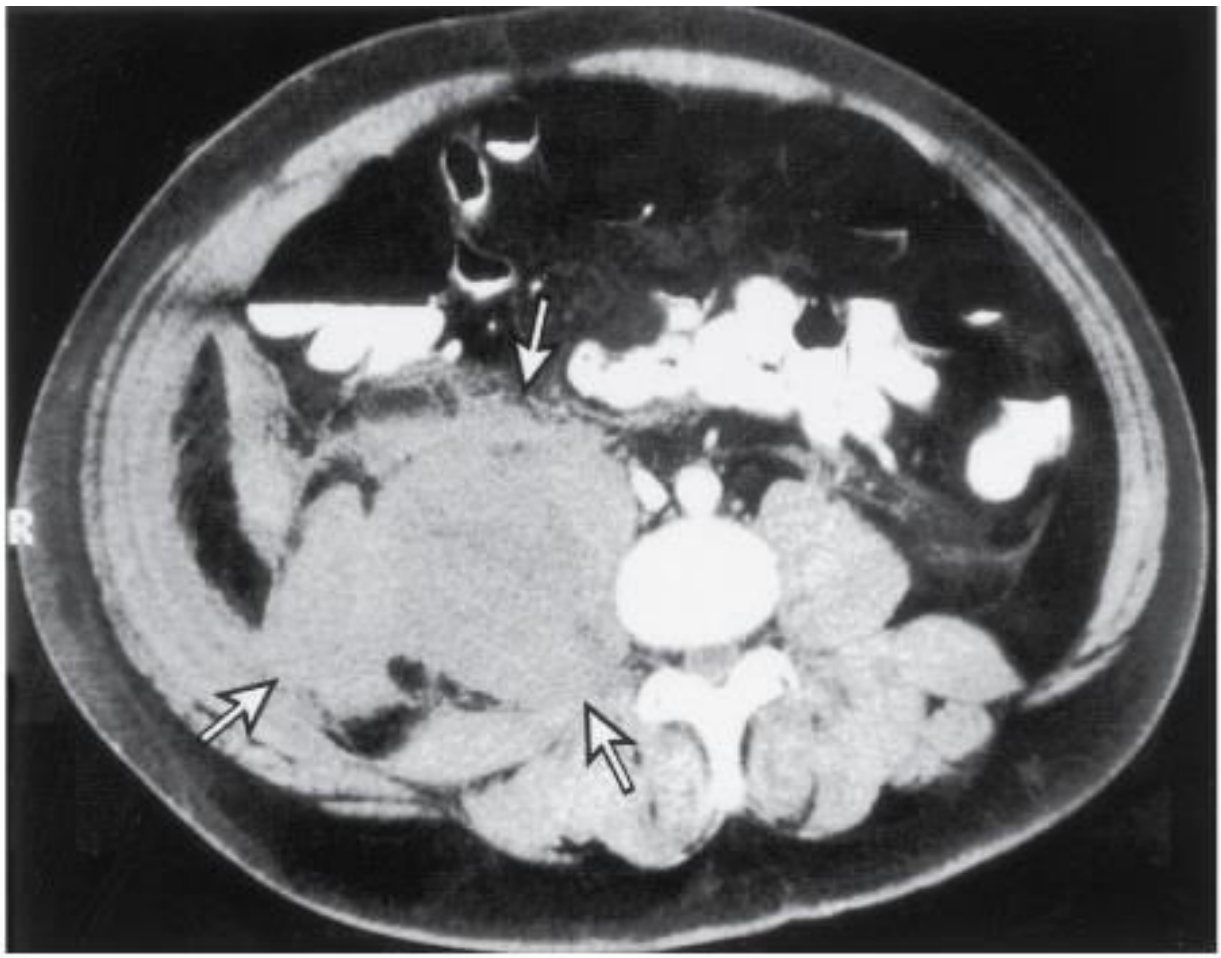
Stage 4 – Prominent bone cysts with marked narrowing of joint space – Fig C (arrows)

Stage 5 – Joint obliteration with epiphyseal overgrowth.

HEMATOMAS:

Hematomas can occur in the subcutaneous plane or into the muscle. They are very characteristic of clotting factor deficiencies. Hematomas, with bleeding into the muscles occur most commonly in the quadriceps, iliopsoas and forearm. Smaller hematomas resorb without any residual defects. If large and in severely affected patients subcutaneous bleeds can dissect into the muscle plane.

Retroperitoneal hematoma:



Source: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology*, 7th Edition: http://www.accessmedicine.com

Retroperitoneal hematomas can dissect through the diaphragm into the chest and neck and compromise the airways. They might cause ureteral obstruction and compromise renal function. An abdominal hematoma can rupture and drain into the colon which is very rare and most often fatal.

Iliopsoas bleeds tend to be large and compromise neurovascular structures causing compartment syndrome. These bleeds can be localised with ultrasound and require larger doses of factor VIII.

Bleeding around airways is an emergency and must be treated immediately with large doses of factor VIII to prevent compromise of the airways.

Untreated hematomas can lead to pseudotumor formation, with the formation of a fibrous membrane around the hematoma.

Patients with Haemophilia can develop hematoma of the bowel wall and present as appendicitis, intestinal obstruction or intussusception. The diagnosis of "pseudo appendicitis" can be made with CT scan.

PSEUDOTUMOR:

Pseudotumor is also known as blood cysts. There are three types of pseudotumor.

Type 1 : Simple cyst; confined by the tendinous attachment within the fascial muscle

Type 2: Simple cyst that compromises the vascular supply to the adjacent bone and periosteum resulting in bone resorption and cyst formation

Type 3: Subperiosteal bleeding resulting in separation of the periosteum from the bone cortex.

Pseudotumors contain either a serosanguineous fluid or brownish material surrounded by a fibrous membrane. They cause pain only when the collection is rapid or when they compress nearby neurological structures. Pseudotumors tend to expand over years together and can become multiloculated. They can reach a stage where they become inoperable. Sinus tract formation from a pseudotumor is a risk for increased infections.

Most common sites for pseudotumor formation is in the lower limb but they can occur at other sites too. Small joints of the hands can be involved in younger patients.

MRI and CT help in diagnosis. Needle biopsy of the pseudotumor should be avoided for fear of bleeding and infection.

The only definitive treatment is complete excision. If incompletely removed the pseudotumor tends to reform.

Pseudotumor of the fibula:

Source: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology*, 7th Edition: http://www.accessmedicine.com

HEMATURIA:

Hematuria is frequently seen in severe haemophiliacs. Colour of the urine can vary from red to brown depending on the rate of the bleeding. Bleeding can occur from anywhere along the genitourinary tract from the renal pelvis till the bladder. Patients can experience colicky pain if clots obstruct the ureter. Bleedings tends to last from days to weeks depending on the severity of the bleed. If the bleeding does not stop within a weeks' time and if the patient is

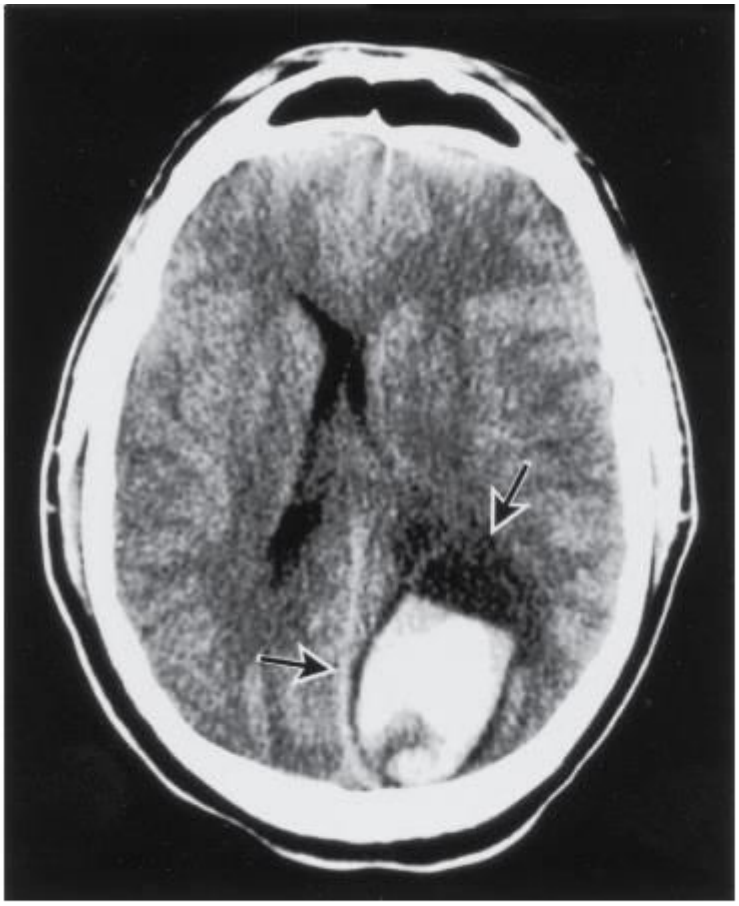
symptomatic, Factor replacement must be given. A search for structural cause of bleeding should also be made if the bleeding is prolonged and does not subside with factor replacement.

NEUROLOGICAL COMPLICATIONS:

Intracranial bleed is the most severe of all complications. The bleed can occur spontaneously but most commonly occurs after a trivial trauma. Symptoms might occur soon after the bleed or it might be delayed. Suspicion of an ICH should arise when a haemophilia patient complains of severe headache. Treatment should be started immediately when there is a suspicion of intracranial bleed without waiting for imaging studies.

Bleeding into the spinal cord is very rare and can result in paraplegia. Epidural bleeding compressing the cord is more common. Muscle hematomas in the periphery can result in peripheral nerve compression. Femoral nerve compression by iliopsoas bleed is most common and can result in sensory loss over the anterior and lateral thigh, weakness and atrophy of the quadriceps. Ulnar nerve is the next most commonly involved peripheral nerve.

Intracranial bleed in severe hemophilics:



Source: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology*, 7th Edition: http://www.accessmedicine.com

MUCOUS MEMBRANE BLEEDS:

Mucous membrane bleeds in the form of epistaxis and hemoptysis is common in haemophiliacs. Peptic ulcer disease is more common in Haemophilia A when compared to the general population.

Occult blood loss in urine or stools might contribute to the iron deficiency seen in these patients and was recorded so in a study conducted in the university of Florida[2].

POST SURGICAL BLEED:

Severe haemophilic patients need to be treated with factor preoperatively and post operatively. Mild or moderately affected patients are at times diagnosed only after bleeding occurs from the surgical site. Wound healing is poor in such patients. Appropriate Factor VIII replacement can prevent intra operative and postoperative bleeding.

Dental extraction is the most common surgical procedure done on haemophilia patients. Loss of permanent teeth causes more bleeding than loss of deciduous teeth.

BLEEDING IN CARRIERS:

A wide range of factor levels have been observed in normal people as well as heterozygous carriers of Haemophilia A or B. Extreme lyonisation can cause excessive bleeding in a female carrier.

Factor levels are independent of the bleeding severity and vary from person to person within the same family. Clotting factor levels should be obtained in all carriers prior to a medical or surgical intervention in order to assess the bleeding risk involved.

COMPLICATIONS IN HAEMOPHILIA

There are three major complications that can occur in haemophiliacs,

- Joint destruction and abnormalities due to hemarthroses
- Blood borne infection transmission
- Development of Inhibitor antibodies

HEMOPHLIC ARTHROPATHY:

There are several factors that contribute to the development of arthropathy. The most important among them would be the deposition of iron in the synovium and the development of synovial fibrosis that leads to contracture formation. The patient has extreme pain and limitation of the range of movement.

Primary prophylactic treatment with factor VIII or IX dramatically reduced the incidence of arthropathy and increased the quality of life. A randomized control trial comparing three times per week prophylactic dosing against the on-demand dosing showed that prophylactic dosing was superior to on-demand dosing schedule.

The relative risk of MRI detected joint damage with episodic therapy as compared with prophylaxis was 6.1 (95% CI 1.5-24)[5]. Orthopaedic complications still remain a major issue as on-demand treatment is still the treatment method followed in India.

The completed Joint Outcome Study in the United States has demonstrated that prophylaxis with Factor VIII at 25-35 units per kilogram body weight every other day is superior to intensive on-demand (eg, 40 units/kg initially, then 25 units/kg at 24 and 72 hours) factor replacement therapy in preventing joint disease in previously pristine joints at age six years. The 80 percent lower incidence of pristine joints in the on-demand arm was confirmed by validated physical exam and radiographic scoring as well as by follow-up magnetic resonance imaging of ankles, knees, and elbows in the 66 children randomly assigned between the two arms of the study. [5].

INFECTION:

The incidence of infection has dramatically reduced with the advent of recombinant products and use of intensive donor screening and virucidal techniques. Patients treated with older factor VIII or IX concentrates are at higher risk of developing Hepatitis B, C and D or HIV infection. Co-infection with HCV and HIV has a bad prognosis as far as the liver derangement is concerned. These people tend to respond poorly to treatment. Since the mid 1980s, no HIV infection has been reported with the use of anti haemophilic factor with advanced virucidal techniques.

Other rare infections that can be transmitted through the use of anti haemophilic factors are parvo B19, Creutz feld Jacob disease and the new variant of CJD. CJD and nvCJD are transmissible spongiform encephalopathies.

DEVELOPMENT OF INHIBITORS:

The most important complication of Haemophilia is the development of inhibitors to factor VIII or IX.

HAEMOPHILIA A AND INHIBITORS:

The development of inhibitors is more common with Haemophilia A than B. The severe Haemophilia A phenotype is most commonly due to a null mutation. A null mutation refers to the complete absence of the protein thereby predisposing to the development of inhibitors. In severe disease 30%, moderate disease 3% and in mild disease 0.3% tend to develop inhibitors.

Genetic and environmental factors play a role. The presence of a first degree relative with Inhibitors increases the risk of inhibitor development three fold in the patient.

Established Risk Factors	Possible [a]
Type (hemophilia A > hemophilia B)	Age at first exposure
Severity (severe > mild/moderate)	Type of factor concentrate (plasma-derived vs recombinant factor VIII)
Underlying mutation (e.g., intron 22)	Method of infusion (continuous vs bolus)
Race (African/Latino > Caucasian)	Prophylaxis vs on-demand
Family history	

Mutations that play an important role in inhibitor formation include, inversion of intron 22, large deletions affecting more than one domain and nonsense mutations involving the light chain. There is a protective factor against inhibitor development when prophylactic treatment is started at an early age. Inhibitor development is seen more in patients exposed to continuous factor infusions as seen during surgeries. As far as the type of factor is concerned, the recombinant factor VIII tends to be more immunogenic when compared to the old low/intermediate purity plasma derived factor VIII. The potential causes for recombinant factor VIII being more immunogenic would be the formation of neo antigens during manufacturing process and the absence of von willebrand factor.

Normally when factor VIII is secreted, it is non-covalently bound to vWF via the light chain, particularly through interactions with *a3* and the C2 domain. Upon thrombin activation, factor VIIIa dissociates from VWF and via the C2 domain, which is no longer bound by VWF, binds to phosphatidylserine on the

platelet membrane. Inhibitors interrupt this process through a number of different mechanisms[1].

With the development of inhibitors, the frequency of bleed does not increase but the patient tends to respond poorly to treatment and develop damaged joints that bleed more frequently. In the worst scenario, a mild Haemophilia patient can become a severe Haemophiliac when the inhibitors react with the reminiscent factor VIII and inactivate them. Any Haemophilia patient who fails to respond to treatment should be evaluated promptly for the development of inhibitors.

The diagnosis is made by using the Bethesda assay. It was developed in 1975 and it depends on the ability of the patient's plasma to inactivate factor VIII in the normal plasma. The result is express

HAEMOPHILIA B AND INHIBITORS:

The incidence of inhibitors in Haemophilia B is less than that in Haemophilia A. The other differences include the possibility of anaphylactic reaction on infusing factor IX concentrates, lesser response to immune tolerance therapy and increased incidence of nephrosis with immune tolerance therapy. Just like Haemophilia B, the incidence of inhibitors increases after factor IX infusion.

Similar to Haemophilia A, inhibitors should be suspected when treatment failure happens. Since anaphylactic reactions are common, patients with high

risk mutations should be screened at regular intervals for the development of inhibitors.

Treatment is similar to that of Haemophilia A with inhibitors. Development of nephritic syndrome during immune tolerance induction is a major concern and regular urine analysis should be undertaken in this subset of patients. The nephritic syndrome that develops responds poorly to steroids making treatment further complicated.

DIAGNOSIS AND DETECTION OF CARRIERS

Diagnosis begins with review of family history especially on the maternal side.

The mother can be identified as a carrier when a family history of bleeding is present. One third of patients have a negative family history. Hence, the lack of family history does not rule out haemophilia.

REASONS FOR NO FAMILY HISTORY:

- The patient might have spontaneous mutations involving factor VIII gene. 25 to 33 % of cases have spontaneous mutations[5].
- Neonatal deaths or the passage of the trait through successive female carriers might give a negative family history[5].

Symptomatic haemophilia is well documented in female. The possible reasons include,

- Unequal and early inactivation of the X chromosome
- Mating between an affected male and a carrier female produces homozygous disease in one half of the female offspring.
- An abnormal karyotype as in Turner's syndrome

1. SCREENING TESTS
2. SPECIFIC ASSAYS
3. DISTINCTION FROM VON WILLEBRAND

SCREENING TESTS:

Three initial tests should be performed in patients presenting with unknown bleeding disorder.

1. Platelet count
2. Prothrombin time
3. Activated partial thromboplastin time

CAUSE OF PROLONGED PT AND OR aPTT:

Test result		Causes of test result pattern
PT	aPTT	
Prolonged	Normal	**Inherited**
		Factor VII deficiency
		Acquired
		Acquired factor VII deficiency
		Mild vitamin K deficiency
		Liver disease
		Warfarin administration
		Inhibitor of factor VII
		Lupus anticoagulant (rare; may be associated with bleeding rather than thrombosis)
Normal	Prolonged	**Inherited**
		Deficiency of factors VIII, IX, or XI
		Deficiency of factor XII, prekallikrein, or HMW kininogen*
		von Willebrand disease (variable)
		Acquired
		Heparin administration
		Inhibitor of factors VIII, IX, XI, or XII
		Acquired von Willebrand disease
		Lupus anticoagulant•
Prolonged	Prolonged	**Inherited**
		Deficiency of prothrombin, fibrinogen, or factors V or X
		Combined factor deficiencies
		Acquired
		Liver disease
		Disseminated intravascular coagulation
		Supratherapeutic doses of anticoagulants
		Severe vitamin K deficiency
		Combined heparin and warfarin administration
		Argatroban with or without warfarin administration
		Inhibitor of prothrombin, fibrinogen, or factors V or X

A normal PT, Platelet count and a prolonged aPTT are characteristic of Haemophilia A and Haemophilia B. The test is abnormal in those with factor levels less than 30%. In mild diseases the aPTT may be normal. So, in case of a mild undiagnosed bleeding disorder with normal lab values factor assays should be done.

Other disorders that prolong the aPTT but not the PT include acquired inhibitors to factor VIII and IX. A similar pattern is also seen in patients with antiphospholipid antibodies but they tend to thrombose rather than bleed.

In the absence of inhibitor which does not occur in patients not treated with the factor, the elevated aPTT should be correctable with normal plasma.

SPECIFIC ASSAYS:

Specific assays for factor deficiency that result in isolated prolonged aPTT are done in the order of statistical significance – VIII, IX and XI. There are two methods to perform the assay for factor VIII – one stage method and two stage method.

The one stage method is preferred as it easier and cheaper. But there are chances of false negatives if only the one stage method was used for diagnosis.

Chromogenic substrate assay is another method for identifying factor VIII levels. The above test depends on the Factor VIII mediated activation of factor X.

Other methods that have developed include, immunoradiometric methods and enzyme linked immunoabsorbent assay.

DISTINCTION FROM VON WILLEBRAND DISEASE:

Ristocetin cofactor assay is the most sensitive test to identify von willebrand disease. It is difficult to perform. Von willebrand is an acute phase reactant and its level increases in times of stress like pregnancy, fever and hormone replacement. In type 2N von willebrand disease, there is a defect in the binding site for factor VIII and bleeding results from the low levels of factor VIII. Type 2N von willebrand is a diagnostic difficulty. It is one differential diagnosis of mild Haemophilia. Type 2N von willebrand disease should be suspected in any female with a low level of Factor VIII.

DIFFERENTIAL DIAGNOSIS

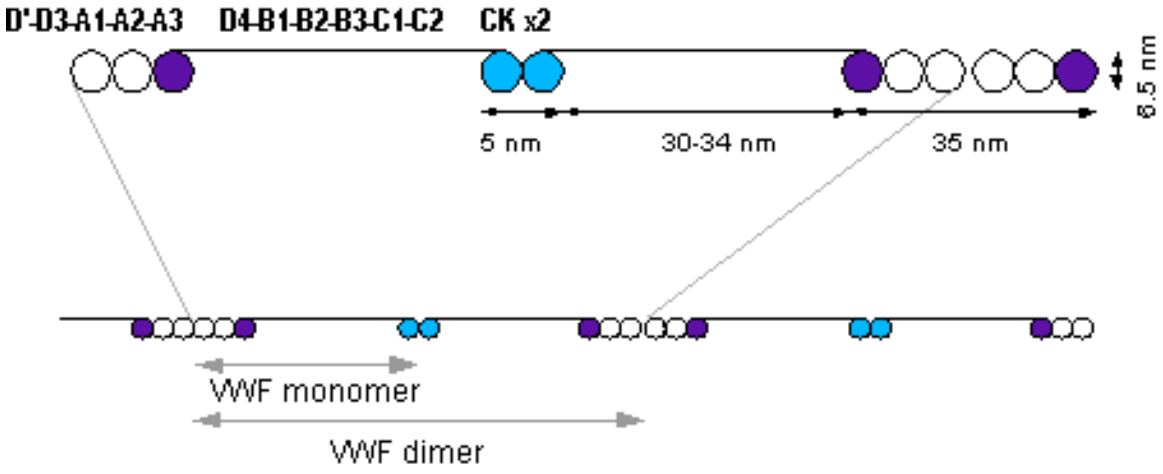
- Von Willebrand disease
- Platelet disorders – eg. Glanzmann thromasthenia
- Clotting factor deficiency – V,VII,X or XI
- Acquired haemophilia

Haemophilia is diagnosed with the presence of a positive family history, prolonged bleeding, hemarthroses and spontaneous soft tissue bleeds. The last two features differentiate it from von Willebrand disease in which hemarthroses and soft tissue bleeds are generally rare except in case of severe type 3 disease. The basic defect in vWd is the reduced activity of von Willebrand factor which acts as a carrier for the factor VIII molecule in vivo. In the absence of the

carrier, the half life of factor VIII is reduced and it is rapidly cleared from the circulation. The main differentiating factors are a prolonged bleeding time, reduced von Willebrand antigen assay and abnormal ristocetin induced platelet aggregation. vWd – Normandy variant is difficult to distinguish from Haemophilia A. In the Normandy variant, vWd levels are normal but factor VIII levels are low. Factor VIII is produced normally but there is a failure in the incorporation of factor VIII into the von Willebrand factor.

Differentiating Haemophilia A and B is impossible from history and physical examination. Factor assay is a must. Similarly, factor assays are needed to differentiate between Haemophilia and deficiency of other clotting factors.

Acquired Haemophilia is seen in autoimmune syndromes, in which inhibitors to Factor VIII develop spontaneously.



TREATMENT

Treatment of Haemophilia includes

- Preventive measures
- Treatment with factor replacement either as on-demand or prophylaxis
- Treatment of complications

PREVENTIVE CARE:

Circumcision:

Approximately 40% of undiagnosed haemophiliacs bleed in association with circumcision. For this reason, male babies born to female carriers should be deferred from the procedure. Whether circumcision can be carried on in this group is still a controversy. Fibrin glue can be used. It reduces the bleeding and the high cost involved in the treatment of haemophilia.

Immunisation:

The routine immunizations given intramuscularly in normal people can be given as deep subcutaneous in haemophiliacs. Smallest gauge needle must be used. Pressure and ice packs must be applied for three to five minutes at the injection site in haemophiliacs. Intramuscular injections are contraindicated in Haemophilia. Hepatitis B vaccine should be given to all infants affected with

Haemophilia. Hepatitis A can be given at one year of age. Inactivated polio vaccine should also be given.

Dental Care:

Proper dental care should be advocated. The patients should be taught about the importance of routine cleaning and maintenance of oral hygiene. Early and proper toothbrush training should be given to the children.

Counselling and Education:

Genetic and Psychosocial counselling should be given to the patient as well as the family members. About 30% of the cases have no family history and in that case proper education about the disease and its complications is very important. Normal socialization and development should be encouraged.

Exercise and Athletic participation:

A regular exercise regimen should be introduced into the life of haemophiliacs. Most of the Haemophilia patients, for fear of bleed, tend to become sedentary and end up with obesity which further increases the risk of bleeds in the weight bearing joints. With the advent of prophylaxis all over the world, The World federation for Haemophilia advocates regular exercise regimen. Proper communication between the parent, patient and staff is very important.

REPLACEMENT THERAPY:

The cornerstone to the management of Haemophilia is the factor transfusion. The dosing is standard but the length of the treatment, choice of product depends on expert individual decision. Guidelines for replacement have been established but the minimum factor level required for haemostasis has not been established. For minor bleeds it's enough if the factor level is raised to 25 to 30% of normal. In case of major bleeds the factor levels should be raised to 50% of normal and in life threatening bleeds and surgery the factor levels should be maintained at 100%. Each unit of Factor VIII per kg of body weight tends to increase the factor level by 2%. Therefore, 1750 units of factor VIII will raise the factor level by 50% of normal. The decrease in factor level post transfusion depends on the pharmacokinetics of the factor. Half life of factor VIII is eight to twelve hours. Half of the initial dose is repeated every eight hours to maintain the desired factor level.

Factor VIII can be derived from the plasma or can be recombinant – derived from cell lines genetically engineered to express large amounts of factor VIII. Plasma products are stratified based on purity and the recombinant products are characterized by their generations. Both can be given intravenously. Solvent treated fresh frozen plasma can be given in the absence of factor and the transmission of viral diseases is very much reduced.

- Intermediate purity concentrates contain 6-10units/mg of protein
- High purity products contain 50 units/mg of protein
- Ultra high purity products include the monoclonal antibody affinity-purified plasma derived concentrate and recombinant factor.

FIRST GENERATION RECOMBINANT FACTOR VIII:

These recombinant factors are derived from the cell cultures of transfected hamster derived cell lines and need no further purification. The human albumin, added for stabilization purposes, contributes to the risk of viral contamination.

SECOND GENERATION RECOMBINANT FACTOR VIII:

This recombinant factor does not contain albumin. Instead, sucrose is added for stabilization. The protein of factor VIII lacks the B domain (mutated protein). The B domain of factor VIII is not needed for coagulation and deleting it increases the stability of the smaller molecule.

THIRD GENERATION RECOMBINANT FACTOR VIII:

These have no added albumin or added protein at the end of preparation.

LONGER HALF LIFE PREPARATIONS:

Factor VIII with longer half life is desirable as it would decrease the frequency of dosing intervals in Haemophilia patients. The two strategies under study for prolonging the half life of factor VIII are – fusion with the Fc portion of immunoglobulin and reconstitution with pegylated liposomes.

Binding of the factor VIII to the Fc portion of immunoglobulin results in binding to the neonatal Fc receptor present on many adult cells. This binding prevents the degradation of factor VIII thereby increasing its half life about 1.5 to 2 fold.

INVESTIGATIONAL STRATEGIES:

A potentially exciting advance is to develop a product that would bind both Factor X and IXa, thereby bringing the two substrates together and bypassing the cofactor function of Factor VIII. This interesting idea is still under study.

The choice of product depends on the purity, safety and cost. Purity and viral safety are utmost important to both, the treating physician and the patient. Ultrapure products are preferred in HIV coinfected Haemophiliacs as they stabilize the CD4 counts.

FACTOR IX PRODUCTS:

In the 1970s and 80s, Prothrombin complex concentrate was used. It was produced by the co-purification of Vitamin K dependant cofactors. This co-purification resulted in the activation of Factor VII to VIIa resulting in an increase in thrombotic complications. PCCs are no longer preferred because of the increased thrombosis risk. Instead, purified human derived or recombinant factors are used.

PURIFIED FACTOR IX:

Chromatographic partitioning and monoclonal antibody affinity purification are the techniques used to purify Factor IX. They are further subjected to viral inactivation processes.

RECOMBINANT FCATOR IX:

It is genetically engineered by inserting the gene for factor IX into a Chinese hamster ovary cell line. It has no added albumin and is safe in the treatment of patients with previously treated and untreated Haemophilia B. Half life of recombinant factor is about 16 to 17 hours.

LONGER ACTING PRODUCTS:

Factor with longer half life are preferred as it would reduce the dosing intervals. As with Factor VIII, binding of factor IX to Fc portion of immunoglobulin increases the half life about 3 to 5 fold. There are other studies investigating the use of Factor IX fused to pegylated liposomes or albumin.

DOSING:

Early treatment of bleeding episodes with appropriate dose of factor will reduce the duration of bleed and prevent further complications. It also reduced the tendency to re-bleed.

Several plasma products are available for raising factor levels. The major disadvantage of plasma is that large volumes need to be infused for maintaining very low factor levels. It is very difficult to achieve haemostasis with plasma infusion. Cryoprecipitate can be used. It contains 80 units of factor in 10ml. The disadvantages are the dosing of factor VIII can only be estimated and the cryoprecipitate has to be stored in a frozen state.

In case of Haemophilia B, in older days, PCC was used. PCC contains vitamin K dependant factors including protein C and protein S. A few of the factors like VII IX and X become activated and increased incidence of thrombotic events have been reported including DIC.

Table 115–4. Doses of Factor VIII for Treatment of Hemorrhagea

Site of Hemorrhage	Desired Factor VIII Level (% of Normal)	Factor VIII Dose[b] (U/kg Body Weight)	Frequency of Dose[c] (every no. of Hours)	Duration (Days)
Hemarthroses	30–50	~25	12–24	1–2
Superficial intramuscular hematoma	30–50	~25	12–24	1–2
Gastrointestinal tract	~50	~25	12	7–10
Epistaxis	30–50	~25	12	Until resolved
Oral mucosa	30–50	~25	12	Until resolved
Hematuria	30–100	~25–50	12	Until resolved
Central nervous system	50–100	50	12	At least 7–10 days
Retropharyngeal	50–100	50	12	At least 7–10 days
Retroperitoneal	50–100	50	12	At least 7–10 days

To achieve 100% factor level, that is, 1u/ml, 3500 units of factor VIII is required. However, the site and the severity of bleed determines the dosing in a particular patient. Factor VIII can be given as infusion. After a loading dose, about 150 to 200 units per hour can be given as infusion. Factor levels can be monitored regularly using venous sampling.

The dose calculation for factor IX is different from that of Factor VIII as the intravascular recovery of factor IX is only 50%. This is probably due to the binding of Factor IX to collagen type IV in the vessel wall. The dose of factor IX can be estimated by assuming that 1 U of factor IX per kilogram body weight increases circulating factor IX by 1 percent of normal or 0.01 U/ml. Thus, to achieve 100 percent of normal (using only highly purified factor IX products) in a severely affected patient, 100 U of factor IX per kilogram body weight should be given as a bolus, followed by half this amount every 12 to 18 hours. Prophylactic therapy can be attempted in Haemophilia B and the dosing is 20 to 40 units/kg twice a week.

HAEMOPHILIA A AND INHIBITORS:

The most important complication of Haemophilia is the development of inhibitors against factor VIII.

Risk Factors:

1. **Disease severity** – 80% of patients with Inhibitors have factor VIII less than 1%
2. **Exposure to factor concentrates** – majority develop inhibitors after exposure <90 days.
3. **Method of purification of Factor VIII concentrate**
4. **Genetic factors :**

   Family history of inhibitors

   Negative association with HLA Cw5 antigen

   Molecular defects: inversion and crossing-over defect in intron 22, gene deletions, and nonsense point mutations resulting in patients without factor VIII antigen.

The inhibitors against factor VIII are antibodies that belong to the IgG4 subclass. Most commonly these antibodies are directed against the A2 and C domain of Factor VIII. Early diagnosis of factor VIII inhibitors is important. The diagnosis is most often made when a patient does not respond to Factor VIII infusions. The inhibitors are detected using a common assay called the Bethesda assay. A mild modification of this is the Nijmegen assay.

HIGH RESPONDERS:

High responders are defined as patients whose inhibitor titre is higher than 10 Bethesda units (BU) at baseline or whose initial inhibitor titre is less than 10 BU but rises to greater than 10 BU after administration of factor VIII. Thus, high responders who are not treated with factor VIII for long periods may have a sustained high level of inhibitor, or they may have a very low to undetectable level of inhibitor until they are challenged with factor VIII[3].

Major bleeding episodes in high responders with initial inhibitor levels <10BU can be treated with high doses of either human factor VIII or porcine Factor VIII. This high will overcome the inhibitors. Though factor eight bypass activity can be used, it is not as reliable as factor VIII and the effects cannot be monitored by a reliable blood investigation.

In major bleeds factor VIII is given in a dose of 10,000 to 15,000 units stat followed by 1000 units per hr infusion with frequent monitoring of factor VIII levels.

In high responders with inhibitor less than 10BU, who experience minor bleeds, the preferred treatment would be factor eight inhibitor bypass activity or Recombinant factor VIIa. The dose of Recombinant factor VIIa is 90 to 120mcg/kg that can be repeated at two to three hour intervals.

Patients with inhibitors >10BU rarely respond to high levels of factor VIII. In this case the treatment of choice would be either recombinant factor VIIa or factor VIII inhibitor bypass activity for both minor and major bleeds.

LOW RESPONDERS:

Low-responder patients are arbitrarily defined as patients whose inhibitor titre is less than 10 BU even after challenge with factor VIII. For major bleeds high dose of factor VIII is recommended. In case of minor bleed, recombinant factor VIIa or FEIBA is preferred as most low responders become high responders when challenged with repeated doses of factor VIII.

IMMUNE TOLERANCE INDUCTION:

The most promising approach to the eradication of inhibitors is the immune tolerance induction. This involves the daily expose of these patients to factor VIII. Both low dose and high dose regimens have been tried. Bleeds that happen during the immune tolerance period are treated with factor VIII inhibitor bypass activity.

Other immunosuppressive drugs, including cyclosporine and rituximab have been tried to eradicate the factor VIII inhibitors. However, these seem more promising in cases of acquired antibodies, in which case the antibodies are autoantibodies rather than alloantibodies as seen in Haemophilia patients.

IMMUNE TOLERANCE PROTOCOL	DOSE	RESPONSE
High dose regimen	100 U/kg factor VIII two times per day until antibody reaches 1 BU/ml, then 150 U/kg factor VIII per day until factor VIII half-life is normal	In 16 of 21 patients, titre fell to <1 BU/ml
Low dose regimen	50U FVIII/kg/day	9 out of 12 responded
Netherlands protocol	25U FVIII/kg/day	11 out of 18 responded

OTHER TREATMENT OPTIONS IN HEMOPIHLIA A:

DESMOPRESSIN:

Desmopressin is effective in mild to moderate haemophilia. Severe Haemophiliacs do not respond. The levels of Factor VIII were found to increase in normal as well and mild and moderate haemophilia after infusion. The dose is 0.3mcg/kg body weight and the factor level increases 30 to 60 min after infusion. A concentrated nasal spray can be used at a dose of 150mcg in each nostril. However, the response to desmopressin should be checked before a

bleed as in certain instances mild and moderate haemophilia patients might not respond. Tachyphylaxis can happen with repeated administration of desmopressin.

ANTIFIBRINOLYTIC THERAPY:

Antifibrinolytics like epsilon aminocaproic acid and tranexamic acid can be used as adjuvant in cases of mucosal bleeds. They are contraindicated in the presence of hematuria. The dose of EACA is 4 to 5gms stat followed by 1gm/hr. Tranexamic acid can be given as 1g every fourth hourly.

FIBRIN GLUE:

Fibrin glue is also known as fibrin tissue adhesive. It contains a mixture of fibrinogen, thrombin and factor XIII and can be applied topically to the injury site. It is most commonly used as an adjunctive to dental procedures.

LIVER TRANSPLANTATION AND GENE THERAPY:

Liver transplantation has been done successfully in patients with Haemophilia and has resulted in complete cure in haemophilic patients.

Gene therapy is under study and so far the results have been poor with the level of factor rise being very less and the level remained elevated only for a month.

FACTOR IX AND INHIBITORS:

When the inhibitor titre is less than 10BU, it can be overcome with high doses of factor IX. In case of acute bleeds in patients with inhibitor levels 5 to 10 BU/ml should be treated with same factors used to bypass the activity of factor VIII inhibitor. Recombinant factor VIIa can be used in the dose of 90 to 120 mcg/kg ever 2 to 3 hrs.

Induction of immune tolerance can be tried with daily dosing of purified factor IX. Anaphylaxis and nephritic syndrome are two major complications. Patients who experience these complications must be treated with recombinant factor VIIa.

GENE THERAPY FOR HAEMOPHILIA B:

One of the interesting approaches to gene therapy for Haemophilia B has been the introduction of an AAV vector containing the cDNA of factor VII, which, when secreted, becomes activated.[122] When factor VIIa is expressed in Haemophilia B mice, even at low levels, the animals experience fewer bleeding episodes. No thromboembolic side effects were noted. Although gene transfer trials for haemophilic patients currently are suspended, ongoing studies of new vectors and in animal models of Haemophilia are encouraging.

MATERIALS AND METHODS

MATERIALS AND METHODS

This study was done at Government Royapettah Hospital, Chennai for a period of six months from April 2014 to September 2014. The study was performed after procuring informed written consent from all the participants involved. Clearance was obtained from the Ethical Committee of the Government Kilpauk Medical College & Hospital Chennai.

STUDY DESIGN:

The study design is a cross sectional study.

POPULATION:

The study population included 50 patients who attended the Haemophilia OP at Government Royapettah Hospital and in-patients in the same hospital.

INCLUSION CRITERIA:

Patients diagnosed as Haemophilia and attending the Haemophilia OP.

Newly diagnosed in- patients in Government Royapettah Hospital.

EXCLUSION CRITERIA:

1. Patients with acute infections
2. Patients with recent blood transfusion
3. Patients with haemorrhoids and portal hypertension

METHODOLOGY:

All patients, diagnosed and registered in the Haemophilia clinic were taken as the study population. The sample size was set to be 50. A detailed history regarding the onset and progression of the disease, family history, maternal carrier status, treatment history and the presence of complications were taken.

After obtaining consent blood was drawn for investigations.

The following investigations were done:

- aPTT
- BT
- CBC
- HIV
- HBsAg
- Anti HCV
- Hb levels

- Ferritin levels
- Peripheral smear

The results obtained were then analysed to identify iron deficiency and the clinic haematological profile in Haemophilia patients.

Serum ferritin concentration is proportional to the amount of iron in the body. It was measured using immunoassay method.

Step 1 – The binding of human serum ferritin to a solid phase antihuman ferritin and the simultaneous binding of the purified antihuman ferritin conjugated with ALP to the insoluble immune complex.

Step 2 – Reaction of ALP with a substrate solution consisting of phenyphosphate disodium and 4-amino antipyrine. Following the addition of potassium ferricyanide a colour develops, the optical density (490-510nm) of which is directly proportional to the ferritin in the sample.

STATISTICAL ANALYSIS

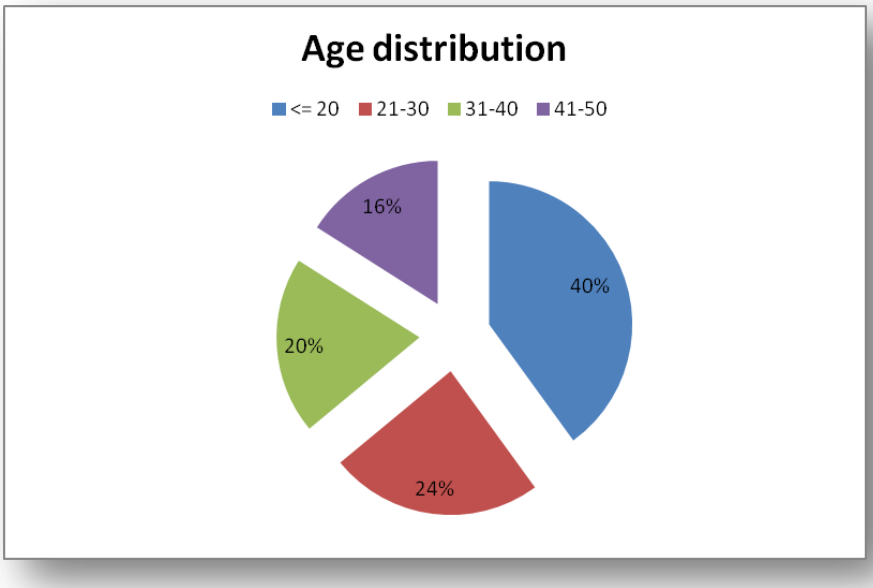
STATISTICAL ANALYSIS

The data obtained was analysed using the SSPS software.

AGE DISTRIBUTION:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	<= 20	20	40.0	40.0	40.0
	21-30	12	24.0	24.0	64.0
	31-40	10	20.0	20.0	84.0
	41-50	8	16.0	16.0	100.0
	Total	50	100.0	100.0	

Age in years



From the analysis, 40% of the patients were less than 20 yrs of age contributing to the highest percentage. 16% of the patients were between the age group of 41 to 50 yrs of age.

The mean age of the study population was 25.28yrs.

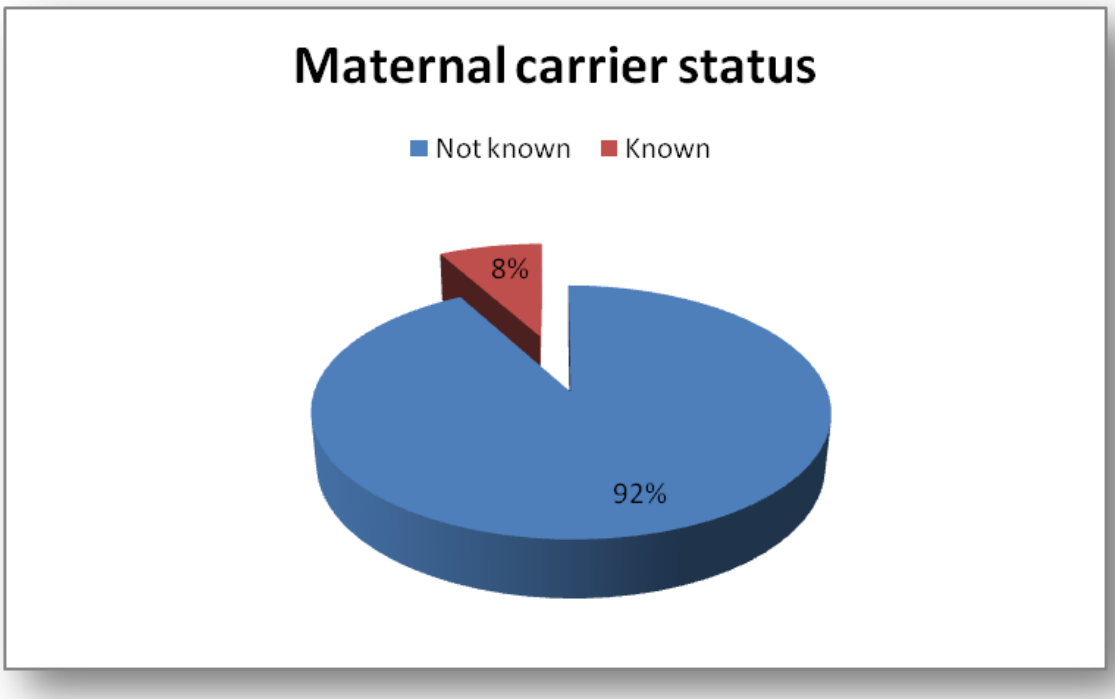
SEX DISTRIBUTION:

All the patients under the study were male. Female haemophiliacs were not identified in the study population.

CARRIER STATUS DISTRIBUTION:

Mother's Carrier Status

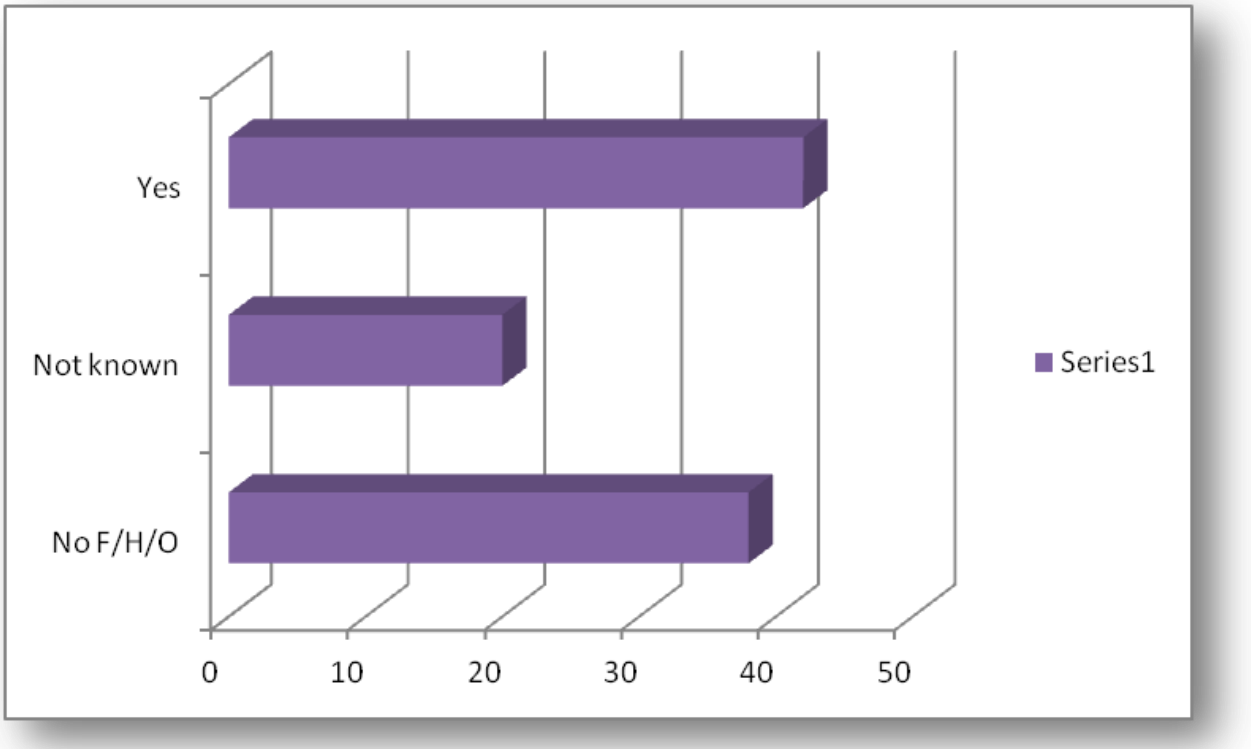
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Not known	46	92.0	92.0	92.0
	Carrier positive	4	8.0	8.0	100.0
	Total	50	100.0	100.0	



Of the study population of 50, only 8% knew the carrier status of their mother. The rest of the 92% were not aware of their maternal carrier status.

FAMILY HISTORY ANALYSIS:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	No	19	38.0	38.0	38.0
	Not known	10	20.0	20.0	58.0
	Yes	21	42.0	42.0	100.0
	Total	50	100.0	100.0	



In the group of 50 haemophiliacs, 30% had no family history. 20% were not aware of their grandfather's or uncle's disease status. 42% had a positive family history either in their maternal grandfather or maternal uncle.

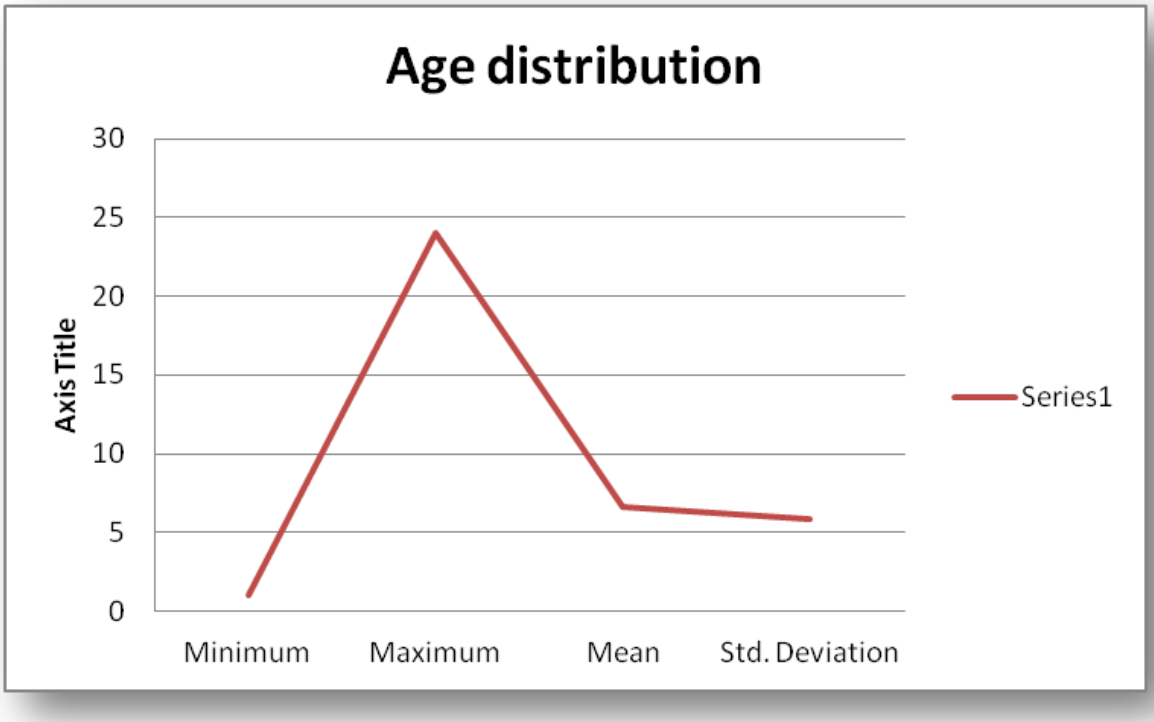
AGE AT DIAGNOSIS:

	N	Minimum	Maximum	Mean	Std. Deviation
Age at Diag	50	1	24	6.58	5.845
Valid N (listwise)	50				

The age at diagnosis was expressed in months and the mean age at diagnosis was found to be 6.58 months.

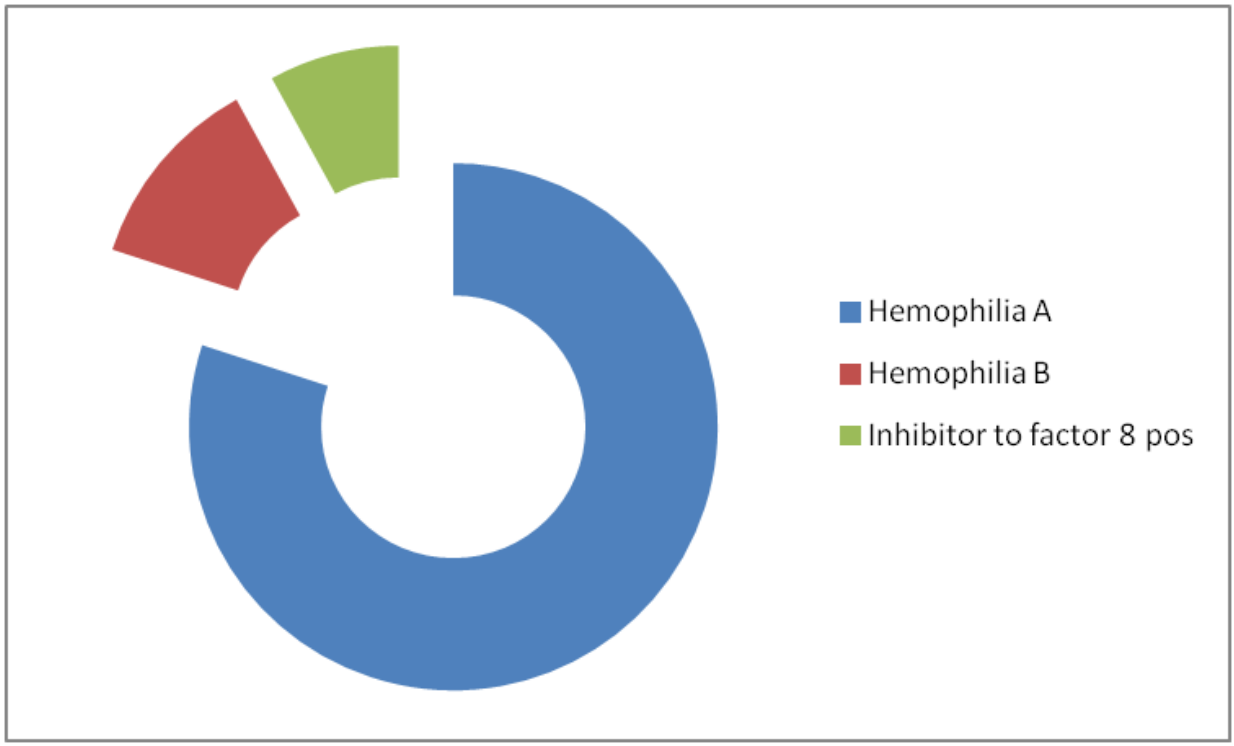
The earliest was at 1 month of age and the latest was at 24 months of age.

The age at diagnosis correlated best with the age of crawling indicating that diagnosis was made when the child was exposed to minor trauma and most of them had a history of purpuric patches on the skin.



HAEMOPHILIA TYPE ANALYSIS:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	A	40	80.0	80.0	80.0
	B	6	12.0	12.0	92.0
	Inhibitor to factor 8 pos	4	8.0	8.0	100.0
	Total	50	100.0	100.0	

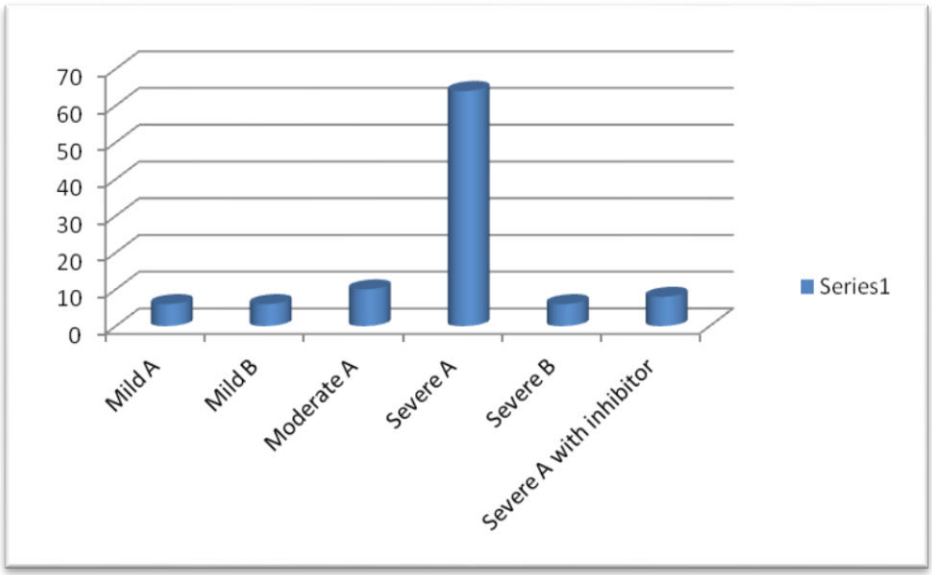


Of the study population

- 80% were Haemophilia A
- 6% were Haemophilia B
- 4% had positive inhibitors to Factor VIII and were severe haemophiliacs

SEVERITY OF HEMOPHILIA:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Mild	3	6.0	6.0	6.0
	Mild B	3	6.0	6.0	12.0
	Moderate	5	10.0	10.0	22.0
	Severe	32	64.0	64.0	86.0
	Severe B	3	6.0	6.0	92.0
	Severe with inhibitor	4	8.0	8.0	100.0
	Total	50	100.0	100.0	



The analysis showed the following results:

- 6% - Mild Haemophilia A
- 10% - Moderate Haemophilia A
- 64% - Severe Haemophilia A
- 8% - Severe Haemophilia A with positive inhibitors
- 6% - Mild Haemophilia B
- 6%- Severe Haemophilia B

TYPE OF TREATMENT:

Type of Treatment

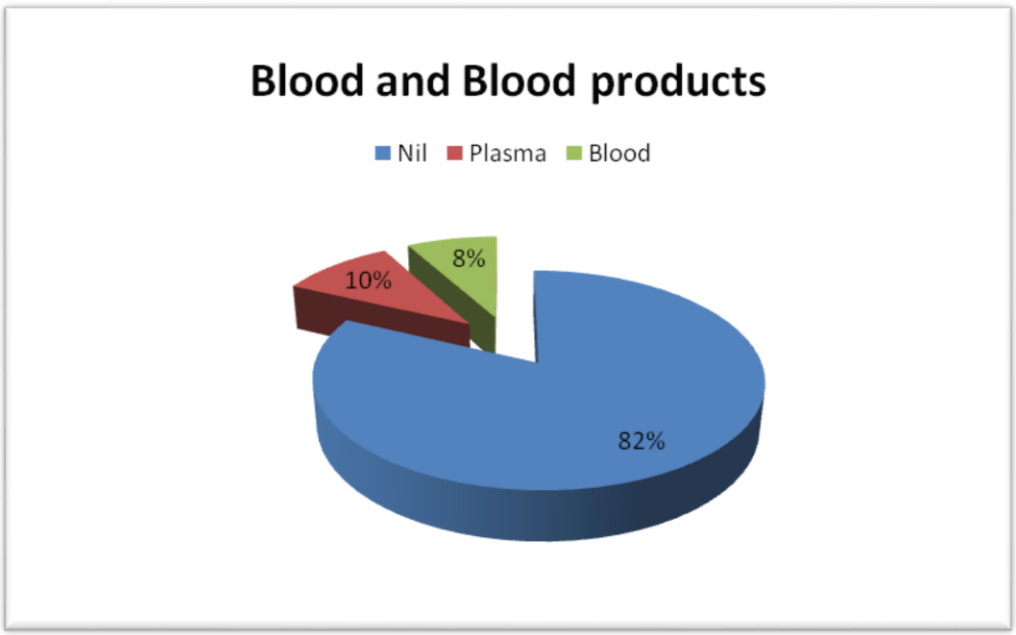
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	On Demand	50	100.0	100.0	100.0

100% of the population were on, on demand treatment with either factor or blood products.

TREATMENT WITH BLOOD AND BLOOD PRODUCTS:

Blood and Bld Products

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Nil	41	82.0	82.0	82.0
	Plasma	5	10.0	10.0	92.0
	Blood	4	8.0	8.0	100.0
	Total	50	100.0	100.0	



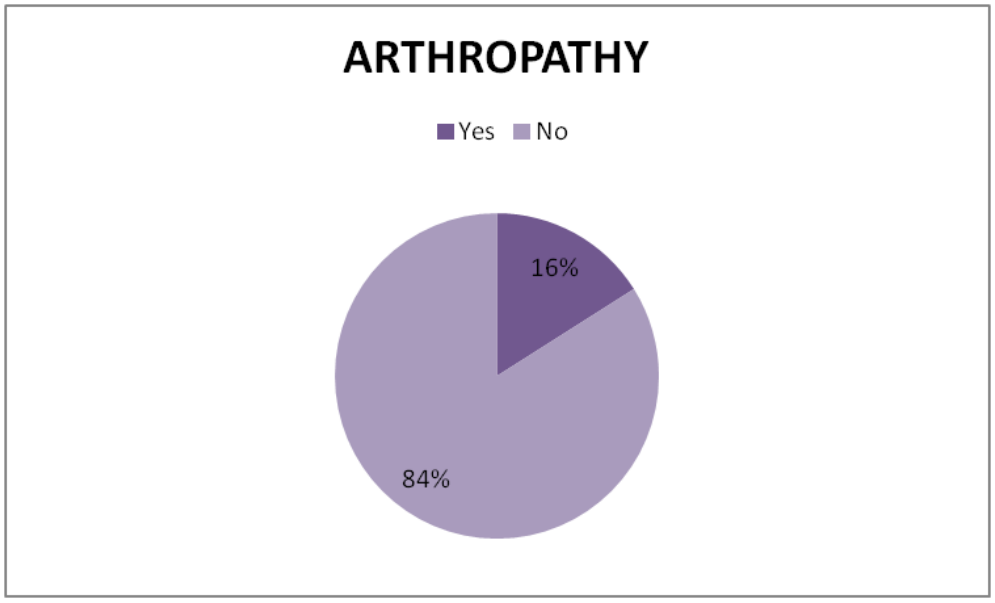
Results of the analysis showed:

- 18% of the study population had received treatment with blood and blood products in their life time
- 82% had received only factor replacement.

DISTRIBUTION OF ARTHROPATHY:

Arthropathy

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	8	16.0	16.0	16.0
	No	42	84.0	84.0	100.0
	Total	50	100.0	100.0	



As per the analysis, of the 50 patients studied, 16% had significant joint deformities. The rest of the 84% had normal range of movements.

The arthropathy occurs due to iron deposition and fibrosis of the synovium. Repeated hemarthrosis can accelerate the arthropathy process thereby affecting the patients' quality of life.

INFECTIOUS COMPLICATIONS:

HIV:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	1	2.0	2.0	2.0
	No	49	98.0	98.0	100.0
	Total	50	100.0	100.0	

HBsAg:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	2	4.0	4.0	4.0
	No	48	96.0	96.0	100.0
	Total	50	100.0	100.0	

HCV:

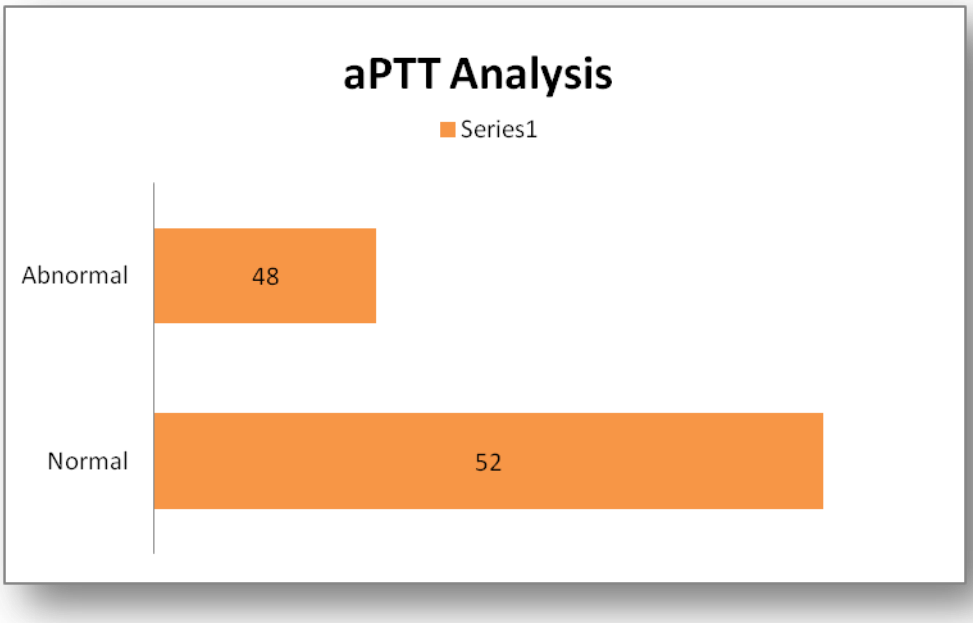
		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	1	2.0	2.0	2.0
	No	49	98.0	98.0	100.0
	Total	50	100.0	100.0	

Of the study population, 2% were HIV infected, 4% were infected with Hepatitis B and 2% were HCV positive. These infections might be due to the usage of blood and blood products in the past.

ANANLYSIS OF aPTT:

aPTT (Sec)

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal	26	52.0	52.0	52.0
	Abnormal	24	48.0	48.0	100.0
	Total	50	100.0	100.0	



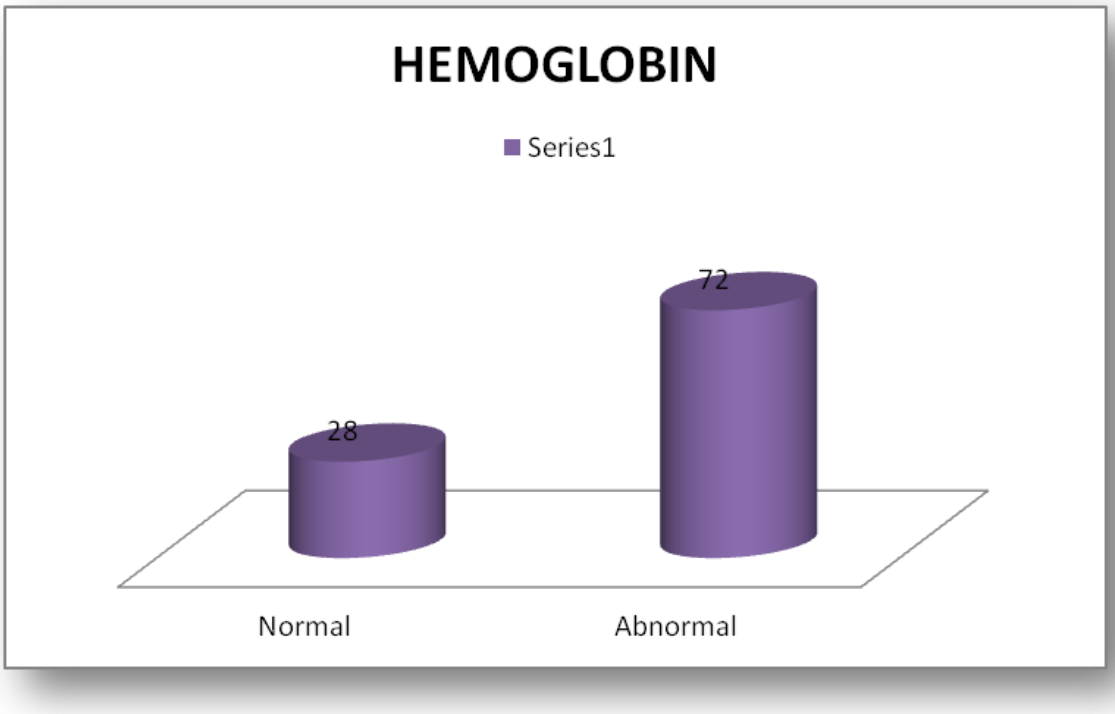
aPTT analysis showed an abnormal value (>40seconds) in about 48% of the study population. The rest of the 52% had normal aPTT values.

The prolonged aPTT signifies a clotting factor defect especially in the intrinsic and the common pathway.

The average aPTT is 54.20 seconds.

HEMOGLOBIN ANALYSIS:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal	14	28.0	28.0	28.0
	Abnormal	36	72.0	72.0	100.0
	Total	50	100.0	100.0	

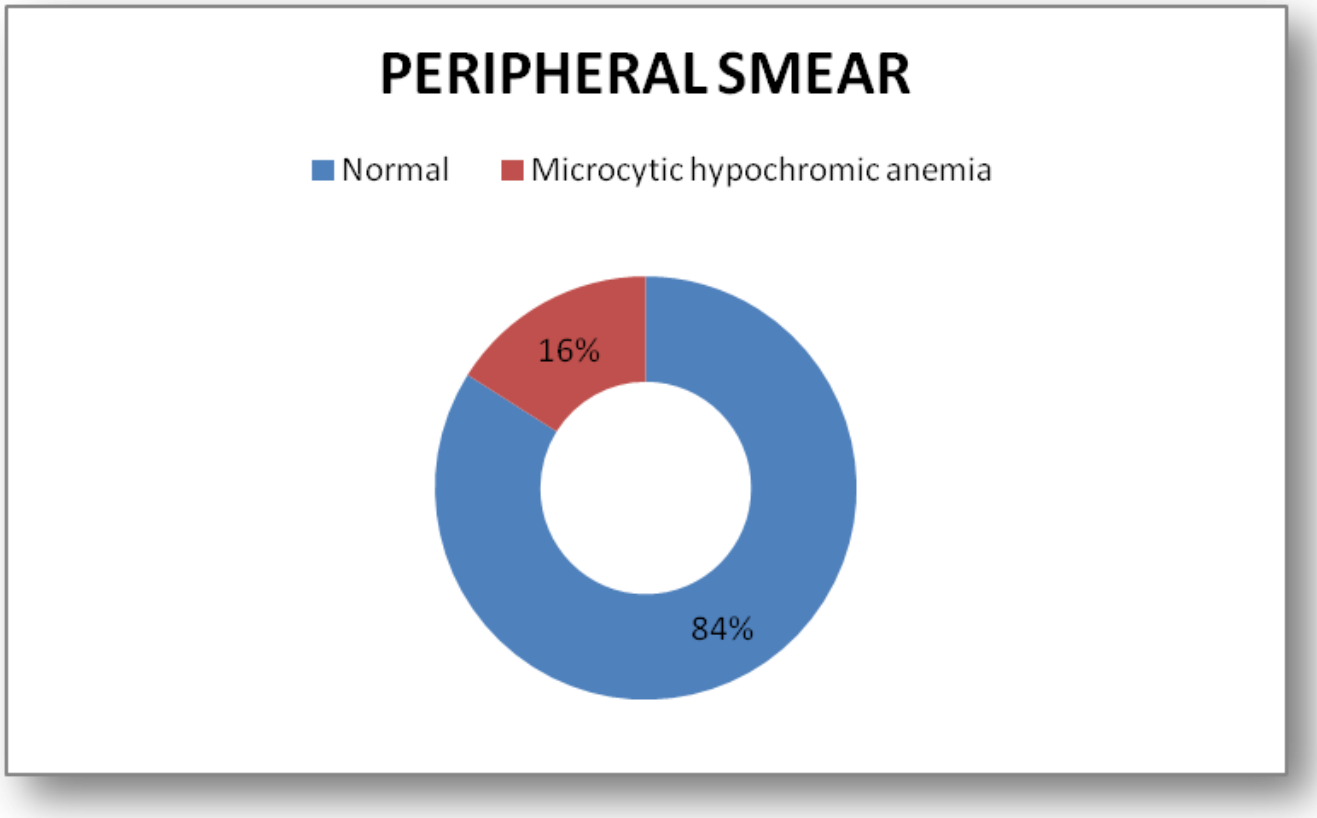


The result analysis showed that 72% had an abnormal Hb level of less than 13mgs/dl. Only 28% had a value greater than 13mgs/dl.

The mean haemoglobin in the study population was 12.18 mgs/dl.

PERIPHERAL SMEAR ANALYSIS:

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Normal	42	84.0	84.0	84.0
	Microcytic hypochromic anemia	8	16.0	16.0	100.0
	Total	50	100.0	100.0	



The results of peripheral smear analysis were as follows:

- 84% had a normal peripheral smear
- 16% had a peripheral smear with microcytic hypochromic anemia picture

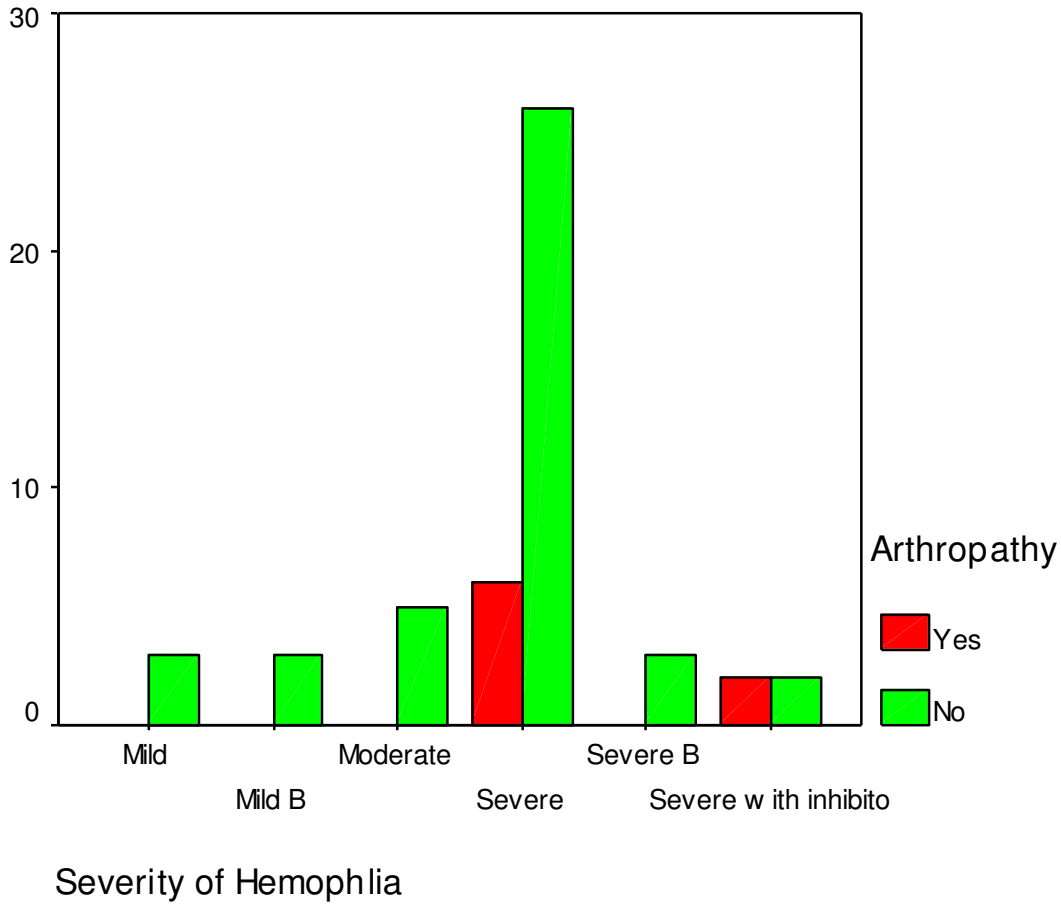
ASSOCIATION BETWEEN SEVERITY OF HAEMOPHILIA AND PRESENCE OF ARTHROPATHY:

Severity of Haemophilia * Arthropathy Crosstabulation

			Arthropathy		
			Yes	No	Total
Severity of Haemophilia	Mild A	Count	0	3	3
		% within Severity of Haemophilia	.0%	100.0%	100.0%
		% within Arthropathy	.0%	7.1%	6.0%
	Mild B	Count	0	3	3
		% within Severity of Haemophilia	.0%	100.0%	100.0%
		% within Arthropathy	.0%	7.1%	6.0%
	Moderate A	Count	0	5	5
		% within Severity of Haemophilia	.0%	100.0%	100.0%
		% within Arthropathy	.0%	11.9%	10.0%
	Severe A	Count	6	26	32
		% within Severity of Haemophilia	18.8%	81.3%	100.0%
		% within Arthropathy	75.0%	61.9%	64.0%
	Severe B	Count	0	3	3
		% within Severity of Haemophilia	.0%	100.0%	100.0%
		% within Arthropathy	.0%	7.1%	6.0%
	Severe A with inhibitor	Count	2	2	4
		% within Severity of Haemophilia	50.0%	50.0%	100.0%
		% within Arthropathy	25.0%	4.8%	8.0%
Total		Count	8	42	50
		% within Severity of Haemophilia	16.0%	84.0%	100.0%
		% within Arthropathy	100.0%	100.0%	100.0%

The analysis were as follows

- Among the patients with mild Haemophilia A and B none had arthropathy.
- None of the patients with moderate haemophilia A have arthropathy.
- Of the patients with severe Haemophilia A , 75% had arthropathy.
- Among the Severe Haemophilia B, none had arthropathy.
- Those who were positive for inhibitors against factor VIII were also severe haemophiliacs and of them 50% had arthropathy.



The arthropathy was majorly grouped in patients with severe haemophilia A, with or without the presence of inhibitors. This might probably be linked to the increased prevalence of joint bleeds in patients with severe deficiency of Factor VIII.

FERRITIN IN THE STUDY POPULATION:

	N	Mean	Std. Deviation	Minimum	Maximum
Mild A	3	54.100	14.8987	37.7	66.8
Mild B	3	51.800	18.2000	32.0	67.8
Moderate A	5	48.200	11.4140	34.0	64.0
Severe A	32	51.091	19.1329	21.0	89.0
Severe B	3	58.167	12.2712	44.0	65.5
Severe A with inhibitor	4	51.150	22.6530	22.0	72.6
Total	50	51.454	17.5141	21.0	89.0

ANOVA

Ferritin (Ng/Ml)

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	214.080	5	42.816	.127	.985
Within Groups	14816.284	44	336.734		
Total	15030.364	49			

Results showed,

- The mean Ferritin in Mild haemophilia A was 54ng/ml.
- The mean ferritin in Mild Haemophilia B was 51.8ng/ml.
- The mean ferritin in Moderate Haemophilia A was 48.2ng/ml.
- The mean ferritin in Severe Haemophilia A was 51.09ng/ml

- The mean ferritin in Severe Haemophilia A with inhibitors was 51.15ng/ml.
- The mean ferritin in severe Haemophilia B was 58.16ng/ml.

ANALYSIS BETWEEN SEVERITY OF HAEMOPHILIAAND FERRITIN LEVELS:

			Ferritin (Ng/Ml)		
			< 50	50-100	Total
Severity of Haemophilia	Mild A	Count	1	2	3
		% within Severity of Haemophilia	33.3%	66.7%	100.0%
		% within Ferritin (Ng/Ml)	3.6%	9.1%	6.0%
	Mild B	Count	1	2	3
		% within Severity of Haemophilia	33.3%	66.7%	100.0%
		% within Ferritin (Ng/Ml)	3.6%	9.1%	6.0%
	Moderate A	Count	3	2	5
		% within Severity of Haemophilia	60.0%	40.0%	100.0%
		% within Ferritin (Ng/Ml)	10.7%	9.1%	10.0%
	Severe A	Count	20	12	32
		% within Severity of Haemophilia	62.5%	37.5%	100.0%
		% within Ferritin (Ng/Ml)	71.4%	54.5%	64.0%
	Severe B	Count	1	2	3
		% within Severity of Haemophilia	33.3%	66.7%	100.0%
		% within Ferritin (Ng/Ml)	3.6%	9.1%	6.0%
	Severe A with inhibitor	Count	2	2	4
		% within Severity of Haemophilia	50.0%	50.0%	100.0%
		% within Ferritin (Ng/Ml)	7.1%	9.1%	8.0%
Total		Count	28	22	50
		% within Severity of Haemophilia	56.0%	44.0%	100.0%
		% within Ferritin (Ng/Ml)	100.0%	100.0%	100.0%

Analysis showed Ferritin levels between 50to100ng/ml in,

- 66.7% of mild haemophilia A patients
- 40% of Moderate Haemophilia A patients
- 37.5% of Severe Haemophilia A patients
- 66.7% of Mild Haemophilia B
- 66.7% of severe Haemophilia B
- 50% of Severe haemophilia with inhibitors

A ferritin level of less than 50ng/ml was seen in

- 33.3% of Mild Haemophilia A patients
- 60% of Moderate Haemophilia A patients
- 62.5% of Severe Haemophilia A patients
- 33.3% of Mild Haemophilia B
- 33.3% of Severe Haemophilia B
- 50% of Severe Haemophilia with inhibitors

The results showed that though the ferritin was within normal value, they were in the lower range of normal. Patients with severe Haemophilia had a much lower ferritin level than those with mild or moderate disease. The results were inconclusive in the Inhibitor group with 50% of the population having low normal ferritin values.

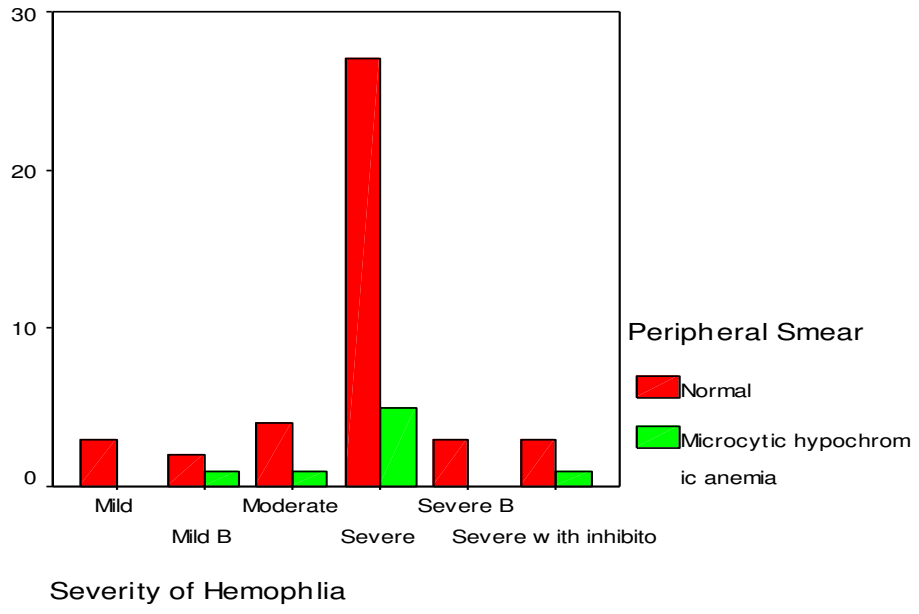
ASSOCIATION BETWEEN SEVERITY OF HAEMOPHILIA AND PRESENCE OF MICROCYTIC HYPOCHROMIC PICTURE IN THE PERIPHERAL SMEAR:

Severity of Haemophilia * Peripheral Smear Crosstabulation

			Peripheral Smear		
			Normal	Microcytic hypochromic anemia	Total
Severity of Haemophilia	Mild A	Count	3	0	3
		% within Severity of Haemophilia	100.0%	.0%	100.0%
		% within Peripheral Smear	7.1%	.0%	6.0%
	Mild B	Count	2	1	3
		% within Severity of Haemophilia	66.7%	33.3%	100.0%
		% within Peripheral Smear	4.8%	12.5%	6.0%
	Moderate A	Count	4	1	5
		% within Severity of Haemophilia	80.0%	20.0%	100.0%
		% within Peripheral Smear	9.5%	12.5%	10.0%
	Severe A	Count	27	5	32
		% within Severity of Haemophilia	84.4%	15.6%	100.0%
		% within Peripheral Smear	64.3%	62.5%	64.0%
	Severe B	Count	3	0	3
		% within Severity of Haemophilia	100.0%	.0%	100.0%
		% within Peripheral Smear	7.1%	.0%	6.0%
	Severe A with inhibitor	Count	3	1	4
		% within Severity of Haemophilia	75.0%	25.0%	100.0%
		% within Peripheral Smear	7.1%	12.5%	8.0%
Total		Count	42	8	50
		% within Severity of Haemophilia	84.0%	16.0%	100.0%
		% within Peripheral Smear	100.0%	100.0%	100.0%

The analysis showed,

- Of the patients with mild haemophilia A, all had a normal peripheral smear.
- There were 3 pts with mild haemophilia B and 1 was positive for a microcytic hypochromic peripheral smear, making it a 33.3% prevalence in the mild Haemophilia B group.
- 20% of the moderate Haemophilia A had an abnormal peripheral smear picture.
- The Severe Haemophilia A without inhibitors included 32 patients. Of them 5 were positive for an abnormal peripheral smear. This is about 15.6% of the severe haemophiliacs.
- Of the severe Haemophilia A with inhibitor 25% had an abnormal peripheral smear.



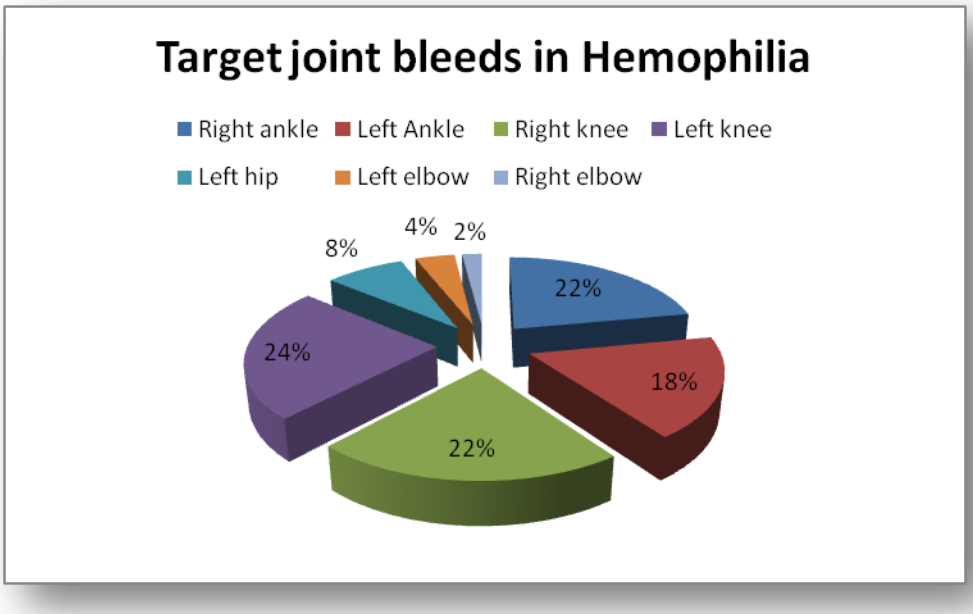
ANALYSIS BETWEEN TARGET JOINT AND SEVERITY OF HEMOPHILIA

			Severity of Haemophilia						
			Mild	Mild B	Moderate	Severe	Severe B	Severe with inhibitor	Total
Target Joint	Right knee	Count	1	2	0	8	0	0	11
		% within Target Joint	9.1%	18.2%	.0%	72.7%	.0%	.0%	100.0%
		% within Severity of Haemophilia	33.3%	66.7%	.0%	25.0%	.0%	.0%	22.0%
	Left knee	Count	0	1	2	6	1	2	12
		% within Target Joint	.0%	8.3%	16.7%	50.0%	8.3%	16.7%	100.0%
		% within Severity of Haemophilia	.0%	33.3%	40.0%	18.8%	33.3%	50.0%	24.0%
	Right ankle	Count	2	0	2	6	0	1	11
		% within Target Joint	18.2%	.0%	18.2%	54.5%	.0%	9.1%	100.0%
		% within Severity of Haemophilia	66.7%	.0%	40.0%	18.8%	.0%	25.0%	22.0%
	left ankle	Count	0	0	0	7	1	1	9
		% within Target Joint	.0%	.0%	.0%	77.8%	11.1%	11.1%	100.0%
		% within Severity of Haemophilia	.0%	.0%	.0%	21.9%	33.3%	25.0%	18.0%
	Left hip	Count	0	0	1	3	0	0	4
		% within Target Joint	.0%	.0%	25.0%	75.0%	.0%	.0%	100.0%
		% within Severity of Haemophilia	.0%	.0%	20.0%	9.4%	.0%	.0%	8.0%
	Left elbow	Count	0	0	0	1	1	0	2
		% within Target Joint	.0%	.0%	.0%	50.0%	50.0%	.0%	100.0%
		% within Severity of Haemophilia	.0%	.0%	.0%	3.1%	33.3%	.0%	4.0%
	Right elbow	Count	0	0	0	1	0	0	1
		% within Target Joint	.0%	.0%	.0%	100.0%	.0%	.0%	100.0%

	% within Severity of Haemophilia	.0%	.0%	.0%	3.1%	.0%	.0%	2.0%
Total	Count	3	3	5	32	3	4	50
	% within Target Joint	6.0%	6.0%	10.0%	64.0%	6.0%	8.0%	100.0%
	% within Severity of Haemophilia	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%	100.0%

Analysis showed that the weight bearing joints – knees and ankles were most commonly affected. Right knee bleed contributed to 22% of the total bleeds in Haemophilia patients. Left knee contributed to 24% of the total joint bleeds in Haemophiliapatients.

Right ankle bleeds contributed to 22% and left ankle bleeds to 18% of the bleeding manifestations. This shows that weight bearing joints are more commonlt affected. The other joints had a minor percentage contribution.



DISCUSSION

DISCUSSION

PREVELANCE AND GLOBAL STAND

The analysis showed that the age group that formed the major group of patients was less than 20 yrs of age. This is probably due to the better diagnosistic facilities available and better awareness among the general public and the carrier parents. Patients betweet 41 and 50 yrs of age contributed a less proportion of the total study population.

Indian data from 2011 showed that there were 14,718 patients with nleeding disorder and of that 11,586 patients were HaemophiliaA[6]. India reports the second largest number in patients with bleeding disorders and the third largest in those with HaemophiliaA[4].

ESTIMATED NUMBER OF PATIENTS

The prevalence of Haemophilia A in India is around 0.9 per 1,00,000 people. This low number of prevalence might reflect the underdiagnosis, under reporting and early mortality of cases. When compared with the developed world the rate of case detection in India is 5 times lesser than that in the developed world.

Using the population data for 2011 from the Census of India15 and a prevalence of haemophilia A of 4 per 1,00,000, the estimated number of haemophilia patients in India would be around 48,407[6].

Haemophilia B has a lower prevalence rate of 0.1 per 1,00,000 population. This shows the need for better surveilance and case reporting in India. The estimated patients with HaemophiliaB would cross 20,000 in number.

With this study incidence and prevalence of the study could not be estimated as its a cross sectional descriptive study.

CASE DETECTION

India has reported the second maximum newly diagnosed cases in the world, in the last one year. However, studies for predicting the future trends in hemophila in India is absent. With better laboratory services and better awareness among both the patients and the doctors, the number of newly diagnosed cases might increase.

Observed estimated and real prevalence of Hemophlillia patients in the various states of India and the union territories have been represented in the following pictorial representation.

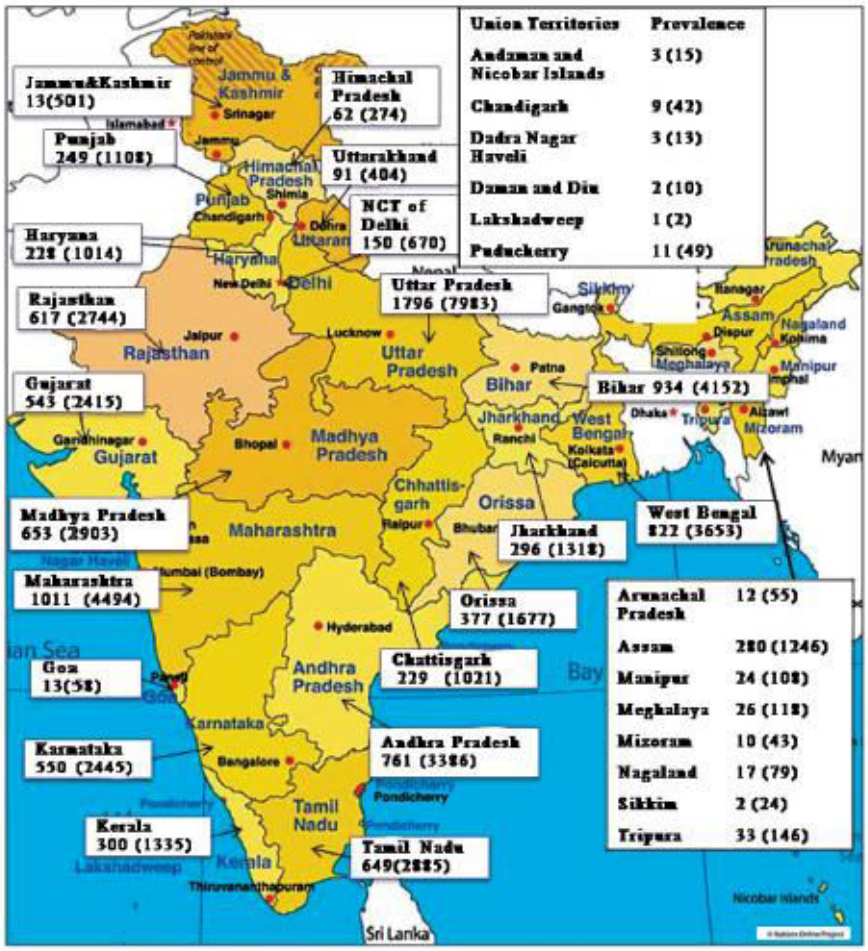


Fig. 4. Observed (calculated at 0.9 per 1,00,000) and estimated (calculated at 4 per 1,00,000 population) prevalence of haemophilia A for States and UT of India. (*Source*: Authors' calculation based on data from Ref. 15).

ORTHOPEDIC COMPLICATIONS

In this study arthropathy was seen in 16% of the study opulation. However, in a study by Kar et al, prevalence of the disability has been estimated. According to this study, of 148 patients with severe Haemophilia A only 9 were free of disability[7]. The orthopedic complications were seen more in those who belonged to the lower socio economic group. Kar et al found the increaseed incidence of fractures in patients with severe Haemophilia on follow up.

In this study follow up was not done and the socio economic status was not analysed. In this study only 16% had arthropathy but all the 16% were severe Haemophilia patients.

TRANSFUSION RELATED INFECTIONS

In remote areas where access to factor replacement is difficult, usage of blood and blood producs can result in the transmission of infections. No specific data is available regarding the usage of blood products by haemophilic patients at the national level. In this study involving 50 patients from our hospital, 10% had received plasma transfusion and 8% had received whole blood transfusion.

The Annual Global Report 2011 showed that 1.12% of Haemophilia patients were HIV positive. There is one large study that shows the prevalence of HIV, HBsAg and HCV in hemophilics. According to that 323 severe and 77 moderate Haemophilia patients had transfusion related infections[4].

In this study, 2% of the 50 were positive for HIV, 2% were positive for HCV and 4% were positive for HBsAg. All those whohad infection were severe Hemophilics. And of those who were infected 50% had a positive history of receiving blood or blood products in the past.

The method of testing used for HCV also had a significance in the outcome. When RT-PCR was used the prevalence was 7.1% and when ELISA was used prevalence dropped to 6.2%[6].

INHIBITORS

Development of inhibitors to factor VIII concentrates is the most dreaded of complications. Factor VIII concentrate is neutralized by the inhibitors. These inhibitors are alloantibodies with high affinity. They belng to the IgG subclass. In this study, 8% were positive for inhibitors and all were severe Haemophiliawith Factor VIII levels less than 1%.

Studies in India showed that the prevalence of inhibitors were found to be 8.3 to 12%[4].

The treatment for inhibitors is Immune tolerance induction. In view of the expense involved in ITI treatment, initiating treatment plans in India is still under question.

TYPE OF TREATMENT

There are two types of treatment in Haemophilia– On demand and Prophylactic treatment. Though prophylactic treatment has a decreased incidence of joint complications, the cost of treatment per patient is high.

Though India is the second largest in the number of Haemophilia patients our per capita usage of factor products was only 0.032 when compared to USA which had a per capita usage of 5.17.

In view of the cost and the inacessebility to Haemophilia treatment centres, Indian patients are still under on demand treatment. Prophylactic treatment is still under study in our population.

JOINT INVOLVEMENT

The weight bearing joints are more commonly affected in Hemophilia. This has been shown in the study by the greater percentage contribution of knee and ankle bleeds. The frequent joint bleeds cause damage and result in arthropathy that can severly affect the quality of patient's life. Most people tend to discontinue their job in view of their arthropathy thereby increasing the economic burden of the family.

FERRITIN AND PERIPHERAL SMEAR

In this study it was found that ferritin levels were in the low normal range in people with severe Hemophilia. Arthropathy was more in the same group of population. The low ferritin can be attributed to the frequent bleeding and hemosiderin deposition in the synovium contributing to the arthropathy.

CONCLUSION

CONCLUSION

Haemophilia is an inherited bleeding disorder with X linked inheritance. Majority of the Haemophilia are factor VIII deficient and have factor levels less than 1%. The genetic make up and the profile of the patients vary in the ethnic groups of India. Prevention of Hemophilic birth is limited in our country due to the absence of prenatal diagnostic facilities. Treatment facilities in our country need to be improved to make the fcators available for the patients belonging to the lower socio economic group.

The results from the analysis is as follows:

- Majority of the sample population were less than 20 yrs of age.
- Female Hemophiliacs (due to disporportionate lyonisation of the X chromosome), were not identified in the study group.
- Only 8% of the patients' were aware of their mother's carrier status.
- Haemophilia A is more common contributing to 80% of the Haemophilia population. 12% were Hemophiliac B.
- Most of the Hemophiliacs had factor levels less than 1% and belonged to the group of severe hemophiliacs.
- 8% of the study population were inhibitor positive.
- A majority of the popultion had a family history of Hemophilia. 20% had no family history.

- The mean age of diagnosis was 6.58 months, the average month when an infant begins to crawl.
- The weight bearing joints were more commonly involved in recurrent bleeding episodes with both the knees having the highest percentage.
- With the advent of plasma derived factors the use of blood related factors have decreased. Despite this 18% had a history of Blood or plasma transfusions in the past.
- Since prophylactic treatment is still under study, all the patients were under on demand treatment.
- Arthropathy, a major complication due to recurrent bleed was seen in 16% of the study population and of the 16% majority were severe hemophiliacs.
- With the use of blood, blood products and plasma derived factors, the prevalence of transfusion related infections can be high. In our group 4% were positive for HBsAg, 2% for HIV and 2% for HCV.
- Majority of the patients had a low Hb of less than 13mg/dl.
- 16% of the patients had a microcytic hypochromic picture on their peripheral smear. The distribution of the abnormal smear was such that, majority were in the Severe Hemophiliacs and the Hemophiliacs with inhibitors.

- The mean ferritin was found to be 51.45ng/dl which is in the low normal range. The mean ferritin was found to be low in moderate and severe hemophiliacs.
- All patients had a low normal ferritin value with the lowest values in the moderate and severe hemophiliac groups.

LIMITATIONS OF THE STUDY

- The sample size was small (50 patients). A larger size would have given better comparable results.
- Short duration of study.
- A scoring system for the arthropathy was not used. Severity of the arthropathy was not graded.
- Serum Iron was not evaluated.
- Analysis of other bleeding manifestations were not done.
- Availability of very few literature for incidence and prevalence calculation.

Further studies involving a larger population is needed for better analysis.

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ANNEXURES

ANNEXURES

PROFORMA

NAME: MOTHER'S NAME:

AGE: CARRIER STATUS:

SEX: PHONE NO:

OCCUPATION:

ADDRESS:

EDUCATIONAL STATUS OF THE PATIENT:

I.BLEEDING

FACTOR DEFICIENT (IF ALREADY KNOWN) :

AGE AT 1ST DIAGNOSIS:

REASON FOR CONSULTATION AT 1ST DIAGNOSIS:

Minor trauma/major trauma/skin bleeds/mucosal bleeds/hematuria/hematemesis/hematochezia/surgery/ dental procedures

FREQUENCY OF BLEED PER YEAR : (Average)

MOST COMMON BLEED SITE:

Joint:

Muscle:

Mucosal:

PREVIOUS HISTORY OF :

1. Joint bleed : Most common:
2. Muscle bleed: Most common:
3. Nose bleed:
4. Skin bleed:
5. Hematuria;
6. Eye bleeds:
7. Intracranial bleed:

II. TREATMENT HISTORY:

HISTORY OF BLOOD PRODUCTS TANSFUSION:

BLOOD PRODUCTS REQUIRED : FFP/Cryo-ppt/Whole blood/Packed cell/Others

HISTORY OF USAGE OF NON BLOOD PRODUCTS:
Desmopressin/ EACA/ Transexamic acid/ other antifibrinolytics/FEIBA/others

TYPE OF TREATMENT : On demand / Prophylactic

III.COMPLICATIONS :

1.Musculoskeletal complications :

- Chronic arthropathy
- Contractures
- Fractures

2.Clnical suspicion of presence of inhibitors:

3.Transfusion related complications :

Hep B/Hep C/HIV/Others

IV.DETAILS OF PREVIOUS HOPSITALIZATION

- No. Of times received Factor:
- Previous hopsitalization:
- No of times hospitalized so far:
- Details of previous Hopsitalization:

INVESTIGATIONS

FACTOR VIII
FACTOR IX
SEVERITY OF HEMOPHILIA:
BT:
CT:
PT:
aPTT:

INHIBITORS:

FERRITIN:

FAMILY HISTORY OF HEMOPHILIA : (Pedigree)

HISTORY OF INHIBITOR POSITIVITY:

S.NO	NAME	AGE	SEX	Mother's carrier status	Family member affceted	Age at diag	Target joint	DEFICIENCY	Levels	Blood and bld products	type of treatment	Arthropathy	HIV	HBsAg	HCV	BT (min)	aPTT(sec)	Hb	Ferritin	peripheral smear	severity of hemophlia
1	Abdul Bashid	14	m	not known	No	8 months	1	A	<1%	no	on demand	no	no	no	no	2	70	13	45	normal	severe
2	Raj Kumar	22	m	not known	No	6 months	2	A	5.13	no	on demand	no	no	no	no	3	45	12.5	54.4	normal	moderate
3	kalyan	30	m	not known	no	24 months	4	B	<1%	no	on demand	no	no	no	yes	2	65	12	65	normal	severe B
4	Prabhakaran	41	m	not known	no	11 months	2	A	<1%	no	on demand	no	no	no	no	1	70	12.6	39	normal	severe
5	Dinesh Kumar	27	m	not known	yes	12 months	4	A	<1%	no	on demand	no	no	no	no	2.15	65	13	45	normal	severe
6	Jai Kishore	7	m	not known	yes	1 month	2	B	5.80%	plasma	on demand	no	no	no	no	3	35	12.3	55.6	normal	mild B
7	jegan	17	m	not known	not known	17 months	2	A	0.90%	plasma	on demand	no	no	yes	no	2	75	13.7	45	normal	severe
8	Dili babu	24	m	not known	no	24 months	2	A	<1%	plasma	on demand	no	no	no	no	3	70	12.7	48.9	normal	severe
9	Hariharan	26	m	not known	no	10 months	4	A	<1%	no	on demand	no	no	no	no	1.45	80	13.7	56.6	normal	severe
10	Natarajan	52	m	not known	not known	10 months	2	A	<1%	no	on demand	yes	no	no	no	0.45	75	11.1	55	normal	severe
11	Purushotaman	31	m	carrier positive	not known	11 months	1	A	<1%	no	on demand	no	no	no	no	2	60	12.2	43.2	normal	severe
12	yuvaraj	25	m	not known	yes	1 month	3	A	7%	no	on demand	no	no	no	no	2	38	13.7	57.8	normal	mild
13	Poojith	14	m	not known	yes	1 month	2	Inhibitor to factor 8 pos	6.2 BU/ml	no	on demand	yes	no	no	no	2	85	13.4	45	normal	severe with inhibitor
14	Anirudh	20	m	not known	yes	1 month	2	A	<1%	no	on demand	no	no	no	no	1.15	63	10.9	21	microcytic hypochromic anemia	severe
15	Hemanth Raj	10	m	not known	not known	12 months	4	A	<1%	plasma	on demand	no	no	no	no	1.3	60	11.9	46	normal	severe
16	Kuralarasu Ce\heran	9	m	not known	yes	1 month	7	A	<1%	no	on demand	no	no	no	no	2	70	12.2	32	normal	severe
17	Adithya	5	m	not known	not known	4 months	1	A	<1%	no	on demand	no	no	no	no	2.15	55	11.6	43	normal	severe
18	Vishal Chandra Jain	22	m	not known	nil	6 months	4	Inhibitor to factor 8 pos	<1%, 71 BU	no	on demand	yes	no	no	no	1.45	75	12.6	65	normal	severe with inhibitor
19	Ramesh Kumar	14	m	not known	no	7 months	3	A	<1%	no	on demand	no	no	no	no	1	45	13.6	76	normal	severe
20	Selavm	35	m	not known	not known	8 months	1	A	<1%	no	on demand	yes	no	no	no	1	55	11	32	microcytic hypochromic anemia	severe
21	Adhikesavan	32	m	not known	not known	7 months	3	A	4%	no	on demand	no	no	no	no	2	42	11.7	45	normal	moderate
22	Arumugam	36	m	not known	no	6 months	2	B	<1%	no	on demand	no	no	no	no	1	53	12.9	44	normal	severe B
23	Venkatesh	34	m	not known	no	24 months	1	A	<1%	no	on demand	no	no	no	no	1	57	13.4	54.6	normal	severe
24	Infant Navin	23	m	not known	yes	1 month	8	A	<1%	no	on demand	no	no	no	no	1	64	11.3	24	microcytic hypochromic anemia	severe

S.NO	NAME	AGE	SEX	Mother's carrier status	Family member affceted	Age at diag	Target joint	DEFICIENCY	Levels	Blood and bld products	type of treatment	Arthropathy	HIV	HBsAg	HCV	BT (min)	aPTT(sec)	Hb	Ferritin	peripheral smear	severity of hemophlia
25	Sampath Kumar	45	m	not known	not known	5 months	7	B	<1%	blood	on demand	no	no	yes	no	2	57	12.6	65.5	normal	severe B
26	Tamizhvannan	22	m	not known	no	7 months	3	A	12.50%	no	on demand	no	no	no	no	1	33	12.5	37.7	normal	mild
27	Kalyanaraghavan	17	m	not known	no	8 months	4	A	<1%	no	on demand	yes	no	no	no	2	67	13.3	46.2	normal	severe
28	Merlin	32	m	not known	yes	1 month	3	A	<1%	no	on demand	no	no	no	no	2.15	57	11.5	77	normal	severe
29	Sai mugilan	19	m	carrier positive	yes	1 month	1	B	6%	no	on demand	no	no	no	no	1.45	35	12.9	67.8	normal	mild B
30	Vadivel	20	m	not known	not known	6 months	5	A	<1%	plasma	on demand	yes	no	no	no	1.3	57	12.7	22.6	microcytic hypochromic anemia	severe
31	Saisiddharthan	9	m	not known	yes	1 month	4	A	<1%	no	on demand	no	no	no	no	2	43	13.1	46	normal	severe
32	Dharmakrishnan	32	m	not known	yes	7 months	3	A	<1%	no	on demand	no	no	no	no	1	47	11.6	35.5	normal	severe
33	Lakshman	5	m	not known	yes	4 months	5	A	<1%	no	on demand	no	no	no	no	1	44	11.8	36.3	normal	severe
34	Shankar	47	m	not known	no	8 months	3	Inhibitor to factor 8 pos	<1%, 10BU	blood	on demand	no	no	no	no	2	62	13.3	72.6	normal	severe with inhibitor
35	Kumaran	31	m	not known	yes	1 month	1	A	<1%	no	on demand	no	no	no	no	2.15	54	12.5	86.5	normal	severe
36	Selvavignesh	18	m	not known	no	8 months	1	A	<1%	no	on demand	no	no	no	no	1	44	13.9	87	normal	severe
37	Praveen kumar	18	m	not known	yes	5 months	2	A	<1%, 16BU	no	on demand	no	no	no	no	1	66	11.1	22	microcytic hypochromic anemia	severe with inhibitor
38	Sadhasivam	46	m	not known	yes	1 month	5	A	<1%	blood	on demand	no	no	no	no	1	43	12.6	43.4	normal	severe
39	Lakshmi narasimhan	10	m	not known	no	8months	5	A	3.50%	no	on demand	no	no	no	no	2	34	13.5	34	normal	moderate
40	Abishek	45	m	not known	yes	1 month	1	A	<1%	blood	on demand	yes	yes	no	no	1	46	11.8	78	normal	severe
41	Srishan	8	m	not known	no	6 months	1	A	17%	no	on demand	no	no	no	no	1	31	12.4	66.8	normal	mild
42	Jagadeesan	24	m	not known	no	7 months	3	Inhibitor to factor 8 pos	2%	no	on demand	no	no	no	no	1.15	36	11.7	43.6	microcytic hypochromic anemia	moderate
43	vijayakumar	31	m	not known	yes	1 month	4	A	<1%	no	on demand	no	no	no	no	1.45	55	13.2	56.2	normal	severe
44	Mohammed zayan	20	m	not known	yes	1 month	3	A		no	on demand	no	no	no	no	1	55	12.6	82	normal	severe
45	ragavan	22	m	not known	yes	6 months	2	A	4%	no	on demand	no	no	no	no	1	40	11.9	64	normal	moderate
46	mohan	45	m	not known	yes	1 month	3	A	<1%	no	on demand	yes	no	no	no	1	47	12.2	45	normal	severe
47	vinodh	50	m	carrier positive	yes	1 month	1	B	6%	no	on demand	no	no	no	no	1	32	11.8	32	microcytic hypochromic anemia	mild B
48	Boosam chengalaih	32	m	not known	nil	6 months	4	A	<1%	no	on demand	no	no	no	no	1	40	13.3	89	normal	severe
49	Venkatraman	19	m	not known	nil	8 months	2	A	<1%	no	on demand	no	no	no	no	1	56	11.9	41.9	microcytic hypochromic anemia	severe
50	Paranthaman	27	m	carrier positive	maternal uncle	7 months	3	A	<1%	no	on demand	no	no	no	no	1.2	57	13.7	56	normal	severe